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UNIVERSITY OF HELSINKI

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From individuals to population – The distribution of Eurasian lynx individuals in space and time and consequences for the local population structure and dynamics

Faculty of Biological and Environmental Sciences
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DOCTORAL DISSERTATION

To be presented for public discussion with the permission of the Faculty of Biological and Environmental Sciences of the University of Helsinki, in Raisio Hall, Forest sciences building, on the 5th of February 2021 at 13 o'clock.

The research in this doctoral thesis was conducted in Natural Resources Institute Finland; Terrestrial Population Dynamics Group, the University of Helsinki; Faculty of Biological and Environmental Sciences Doctoral Programme in Wildlife Biology Research, and in close collaboration with Norwegian Institute of Bioeconomy Research; Division of Environment and Natural Resources.

Supervisors: Katja Holmala and Janne Sundell
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Opponent of the dissertation: Barbara Zimmermann

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When you talk, you are only repeating what you already know. But when you listen, you may learn something new.

Dalai Lama

ABSTRACT

Large terrestrial carnivores are capable of long dispersal distances and thus have a potentially high rate of gene flow between populations. Even with such high mobility, discontinuous habitat and human-caused mortality may constrain dispersal and gene flow. Therefore, isolation of populations because of habitat fragmentation may cause genetic structuring in them because of genetic drift. In a continuous population, geographic barriers should not significantly affect dispersal and gene flow, so the effects of social, ecological and evolutionary forces are easier to detect.

In large carnivores, males generally disperse more often and earlier than females and their dispersal distances are longer than those of females. The direction of sex-bias in dispersal is commonly explained by inbreeding avoidance, polygynous mating systems and male-male competition. Remaining in, or near, the natal home range is explained by kin selection and inclusive fitness. Molecular evidence reveals the spatial genetic structure and clustering of relatives and family lines that may underlie these traits.

We studied the spatial genetic relatedness, family structure, movement patterns and sex- bias of dispersal in the Eurasian lynx (*Lynx lynx*) in an unfragmented population in southern Finland. We used GIS and resource selection functions to analyse telemetry data of dispersing lynx and genetic methods to analyse data obtained from hunted lynx. Dispersal onset age, duration, distance, route or route linearity did not differ statistically between males and females that dispersed. However, the small number of females and the high variation in all dispersal parameters likely affected the outcome of analysis. Linear distance between the start and the end comprised only 20 % of the total dispersal route. Lynx selected their habitat non-randomly. During daylight hours lynx were more discerning in their habitat selection, while most of the traveling took place at night, reflecting the crepuscular and nocturnal activity of the lynx.

According to the results of genetic analyses, the majority of females stayed close to their natal home range after reaching independence. Males dispersed and settled randomly in space. This led to genetic differentiation and spatial clustering of related females but not of males.

Females form the backbone of the local populations, and genetic evidence is in line with the idea that females facilitate the settling of related females. In contrast to females, for males, relatedness is inversely important to avoid inbreeding. Hunting of adult lynx may disturb the forming of matrilineages and decrease genetic variation. Hunting should aim at mimicking a natural mortality pattern, which means hunting mostly young lynx, as the natural adult survival in Eurasian lynx is high.

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CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications:

I Annika Herrero, Juha Heikkinen, Katja Holmala
Movement patterns and habitat selection during dispersal in Eurasian lynx. Mammal Research 14.5.2020.

II Annika Herrero, Cornelya F. C. Klütsch, Katja Holmala, Simo N. Maduna, Alexander Kopatz, Hans Geir Eiken, Snorre B. Hagen
Genetic analyses indicate male dispersal and female philopatry in Eurasian lynx in Finland. Manuscript version 19.5.2020

III Katja Holmala, Annika Herrero, Julia Schregel, Hans Geir Eiken, Alexander Kopatz, Snorre B. Hagen
Genetic evidence of female kin clusters in a continuous population of a solitary carnivore, the Eurasian lynx. Ecology and evolution, 8(22), pp.10964-10975.

The publications are referred to in the text by their roman numeral.

CONTRIBUTIONS

Major author contributions to the original articles.

	I	II	III
Original idea	AH, KH	AH, CK, KH, SBH	KH, SBH, AH, JS, AK, HGE
Data gathering	AH, KH	AH, KH	KH
Data analysis, result interpretation	AH, JH	CK, SM, SBH, AH, KH, HGE	JS, AK, SBH
Manuscript preparation	AH, KH, JH	AH, CK, KH, SBH, HGE, SM, AK	KH, AH, SBH, JS, AK, HGE

1 INTRODUCTION

A species' social structure determines some of the preconditions of home range acquisition, the right to overlap with other home ranges or to be excluded. In part it determines whether one shall leave the natal home range after reaching independence and whether one can be considered a mate, and the way all social contacts are organized. Sociality has been thought to evolve from asociality (Kappeler et al. 2002), when the advantages of living in groups have overridden the costs. These advantages include shared vigilance and other anti-predator behaviour and partitioning of scarce resources or more efficient feeding (Rubenstein 1978). Eurasian lynx (Lynx lynx) are considered a solitary species. At least 85 % of species in the mammalian order Carnivora lead solitary lives (Holecamp and Sawdy 2019). At their trophic level, it is expected that intense competition for food might outweigh the positive effects of living with close kin (Holecamp and Sawdy 2019). Solitary species were thought to lead lives that were just that –solitary, but growing evidence of social contacts beyond reproductive period, aggregation of individuals, complex matrilineal societies and even reciprocity (Quaglietta et al. 2014, Elbroch et al. 2017, Holecamp and Sawdy 2019) are forcing us to reconsider this belief. Solitary is the opposite of gregarious, not the opposite of social (Charles-Dominique 1977).

1.1 CLUSTERING IN SPACE

Molecular evidence reveals spatial genetic structure of populations and, for example, clustering of relatives and family lines, usually of females in mammals (Storz 1999). The formation of such clusters requires a system of kin-discrimination by associative learning (Waser and Jones 1983, Perrin and Lehmann 2001) and a motivation to stay close to relatives. Genetic clustering is common in gregarious, but also in solitary mammals, betraying presence of underlying social structures (Waser and Jones 1983, Goudet et al. 2002, Cutrera et al. 2005). Therefore, it seems as sociality needs to be re-evaluated on a continuous scale rather than as an either-or phenomenon (Waser and Jones 1983, Quaglietta et al. 2014).

The clustering of relatives may lead to increased population density through overlap of individual home ranges (Kamler et al. 2013, Bailey 1993, Aronsson 2017). Clustering may also happen for reasons other than relatedness, such as food distribution pattern or even hunting (Schenk et al. 1998). Also, kin clusters may not be clustering spatially as compared to non-kin (Schenk et al. 1998). This was evident in a black bear population, where all

females irrespective of their relatedness, were highly clustered in space, contrary to their usual matrilinear spacing system (Schenk et al. 1998).

1.2 MAN AND LARGE CARNIVORES

Large carnivores have a predominantly meat-filled diet and require large home ranges to fill their energetic needs (Carbone et al. 1999, Treves and Karanth 2003). In an agrarian human society, prevalent in Europe until the mid-20th century, large carnivores competed with humans for the same resources of wild ungulates, sometimes depredated domestic animals and were seen as a potential threat to human safety (Ericsson et al. 2010). As a consequence, only a hundred years back, humans had eradicated large carnivores from most places in Europe (Chapron et al. 2014).

In the 20th century, ungulate populations grew substantially, partly because of artificial feeding (Festa-Bianchet 2003), and intrinsic value in wild animal populations was extended to include carnivores. It was, perhaps, this change of viewpoint, plentiful ungulates to feed on and human urbanization that allowed the carnivores to gradually inhabit some of the areas they used to populate before (Chapron et al. 2014). This has not, however, taken place without conflict, to which hunting is usually seen as the mitigation tool (Treves and Karanth 2003, Stoner et al. 2006, Peebles et al. 2013). While some aspects, such as the effects of hunting on the age and sex structure of a population, are quite well understood, others remain less known. These include for example the effects of hunting on the social structure and behaviour of the remaining individuals and their effect on population growth (Frank et al. 2017). Large carnivore populations are often substantially structured both genetically (Rutledge et al. 2010) and socially (Rutledge et al. 2010, Leclerc et al. 2017), which selective hunting practices and hunters' preferences may break (Festa-Bianchet 2003, Rutledge et al. 2010, Frank et al. 2017) with unknown or hard to predict consequences (Stoner et al. 2006).

Large carnivore recolonization conveys their incredible ability to adapt to changing environments –a prerequisite of any species to thrive in a changing world. The Eurasian lynx in Europe makes no exception to the history of the larger species. Although dependent on forest cover (Niedziałkowska et al. 2006, Podgórski et al. 2008, article I), according to studies, the lynx is indeed not a species of only mature forests (Sunde 1998, Podgórski et al. 2008, article I). In fact, reasonable anthropogenic impact such as forestry and agriculture creates optimal habitats for viable prey populations in the form of mixed, young forest, sapling stands and fields, and hence enable lynx to find and catch prey and to hide (Podgórski et al. 2008, Filla et al. 2017, Gehr et al. 2017, article I). In this sense, the lynx may, in a way, profit from humans. Large carnivores, including the Eurasian lynx, can be viewed as umbrella or flagship species (Mengüllüoğlu et al. 2017). In order to conserve the lynx, we must

conserve, or perhaps rather manage, large areas of its habitat which also benefits many species on the side (Mengüllüoğlu et al. 2017). In the boreal ecosystem, the Eurasian lynx is a disproportionately important predator in its ecological role than its size suggests, comparable perhaps to that of the African leopard (*Panthera pardus*) or puma (*Puma concolor*) in North America. The Eurasian lynx is an apex predator, physiologically a mix of a medium-sized and a large feline (Viranta et al. 2016), capable of taking down prey four times the size of itself. It has the potential to limit both prey, especially the roe deer (*Capreolus capreolus*), and mesopredator abundance, which has helped in the recovery of other species despite their status also as potential lynx prey (Elmhagen et al. 2010, Richie et al. 2012).

In Central Europe, it is now the habitat fragmentation that sets the boundaries on space acquisition for the Eurasian lynx, the direction of movement and success of finding a vacant home range and a mate (Schmidt et al. 2011, Krojerová-Prokešová et al. 2018). Natal dispersal may end frustrated, and thus prospective mates may only be available from the immediate family (Krojerová-Prokešová et al. 2018). A poacher may create a vacant spot to be inhabited by the next target (Heurich et al. 2018). The outcome of fragmentation and hunting may impede some of the most central life history traits for an individual and threaten the existence of some Eurasian lynx populations. Contrary to Central Europe, Finland has a continuous forest corridor extending to as far as Siberia in the east and the Baltic countries in the south (Ratkiewicz et al. 2014). Consequently, the Eurasian lynx population in Finland is continuous with the Russian and the Baltic lynx populations, without known geographical barriers and thus remains largely unfragmented (Hellborg et al. 2002, Rueness et al. 2003, Ratkiewicz et al. 2014).

After a near-extinction in the first half of the 20th century, the Eurasian lynx have recolonized Finland from east to west. Nowadays lynx occur in the whole country, with most of the population being south of the reindeer husbandry area, which roughly lies between 64˚ and 70˚ North (Luke 2019).

As with any large carnivore, the coexistence between Eurasian lynx and humans does not always go well. In areas with wild-ranging livestock, the problem is lynx predating on domestic or semi-domestic animals (Odden et al. 2006, Walton et al. 2016). These include sheep in most of Europe, but also semi-domestic reindeer in Scandinavia and Finland. The social approval of lynx is rather high among all citizens (WWF 2013). Lynx are responsible for few damages (between 500 – 900 instances, worth approximately 1.1 million € annually between 2010 -2018, the majority of which were damages to semi-domestic reindeer) (MMM 2019). In Finland, the main conflict arises from sharing prey with hunters and the sheer dislike of predators or the perceived threats they pose (Liukkonen et al. 2006). The Ministry of Forestry and Agriculture alleviates these damages and attitudes with a yearly quota to hunt lynx. Lynx population estimation depends on identifying family groups from snow tracks, done by volunteer large carnivore contact persons. The population estimation is based on scientific evidence of adult female minimum

home range size, spacing patterns and habitat characteristics, but the hunting quota is agreed upon by officials, complying with definitions of policy upon large carnivores. Females with cubs are principally protected from hunting, but the outcome is not surveilled by authorities.

1.3 LOCAL POPULATION FORMATION IN LYNX SPECIES

1.3.1 THE LYNX GENUS

Four lynx species are included in the *Lynx* genus. The bobcat (*Lynx rufus*) and the Canada lynx (*Lynx canadensis*) live in North America and have a partly overlapping distribution in the northern United States and southern Canada (Sundquist and Sundquist 2002) on their respective northern and southern distribution ranges. In Europe, the Iberian lynx (*Lynx pardinus*) inhabits the southern Iberian Peninsula and is endangered (Rodriguez and Calzada 2015). The Eurasian lynx has the widest distribution of all *Felidae* or cat species in the world, from mainland Europe in the west to the Pacific coast in the east, from Northern Scandinavia in the north to Nepal in the south (Breitenmoser et al. 2015). While three of the four lynx species are strict seasonal breeders, bobcats have a repeated estrus similar to that of domestic cats and can have young when resources are favourable (Jewgenow et al. 2014).

1.3.2 HOME RANGE ARRANGEMENTS

All the lynx species are predominantly territorial and only resident adults breed (Poole 1995, Krojerová-Prokešová et al. 2018). Female home ranges are generally smaller than male home ranges, which may encompass those of several females' (Lawhead 1984, Litvaitis et al. 1986, Palomares et al. 2001, Herfindal et al. 2005, Cochrane et al. 2006). The territoriality of the Canada lynx is bound to its' cyclic prey species, the snowshoe hare (*Lepus americanus*) causing territoriality to fade out when food is scarce (Poole 1995). For bobcats, a relaxation in territoriality has also been documented (Knick 1990) but is proposed to happen when prey is unlimited (Cochrane et al. 2006).

Home ranges for the *Lynx* species are established in areas with a combination of habitats with adequate prey base (Litvaitis et al. 1986, Aubry 2000, Palomares et al. 2001, Herfindal et al. 2005) and habitat types that are also favoured by the main prey species (Litvaitis et al. 1986, Lovallo and Anderson 1996, Palomares et al. 2001, Vashon et al. 2008, Schmidt 2008). In Russia, Eurasian lynx were noted to migrate up and down mountainous areas between seasons, following roe deer and hares to areas with less snow (Heptner and Sludskii 1992). It is however, unclear whether this happened

within an individual's home range or whether the land tenure relaxed for the winter months, as similar behaviour has not been detected in Eurasian lynx in other areas with migratory prey (Danell et al. 2006, Walton et al. 2016).

1.3.3 PLASTICITY OF HOME RANGES THROUGH TIME AND SEASON

The time an individual has resided in its home range (residence time) may affect home range size differently for each sex (Conner et al 1999). Rural bobcat males tended to increase the size of their home range with longer residence time, while female home ranges decreased (Conner et al 1999). In an urban environment, however, home range size increased with bobcat age, as did overlap (Young et al. 2019). The difference in the outcome through time can be explained by differences in life history strategies and between habitats. For bobcats and Eurasian lynx, dominant males may want to increase their home range size in order to increase their mating opportunities (Conner et al 1999, Mattisson et al. 2013), but the opposite has also been detected (Aronsson et al. 2016). Females, on the other hand, are strongly affected by home range quality (Litvaitis et al 1986, Lovallo and Anderson 1996, Aronsson 2016), but also by the familiarity with their home range (Conner et al 1999). Experience in local knowledge together with increased hunting skills may lead to decreased home range size because of reduced hunting distances (Conner et al 1999). Therefore, a population that consists mostly of mature individuals could have a denser female population and more dispersed males (Conner et al 1999, Vashon et al. 2008). This in turn, may result in differences in local social organization compared to an area with a younger age structure (Conner et al 1999). In the urban population, bobcat home ranges overlapped mostly on the natural environments, which may be imperative for survival in urban areas and perhaps a result of reduced dispersal possibilities (Young et al. 2019).

There are few studies on the stability of (adjacent) home ranges, i.e. what happens when a home range becomes vacant (Lovallo and Anderson 1995, Benson et al 2004). Bobcat females can shift their home range after a death of a neighbour to access a more productive habitat, or upon the arrival of another female (Lovallo and Anderson 1995, Benson et al 2004). Males were found to have aggressive encounters at the time a home range was newly accommodated, probably because of the transitional state the home range was still in, perhaps missing enough olfactory cues of its occupation (Anderson 1998, Benson et al 2004).

The mating season is known to affect home range arrangements in lynx. Reproducing bobcat females in their northern range shifted their home range between seasons whereas males merely expanded theirs (Lovallo and Anderson 1996). Shifts and expansions after the denning time have also been noted for Eurasian lynx females (Schmidt et al. 1997, Aronsson et al. 2016). In a Norwegian study, Eurasian lynx males were observed in aggressive interactions even resulting in death during the mating season, sometimes

resulting in home range takeover by the winner (Mattisson et al. 2013). Male Eurasian lynx home range size decreased during the mating season in Sweden and Norway (Aronsson 2017), which is surprising given the polygynous mating system in which their home range usually encompasses that of several females (Schmidt et al. 1997, Breitenmoser-Würsten et al. 2007). The decrease in home range size may be a spatial and temporal response when home range exceeds a certain size as the males stay guarding receptive females (Sandell 1989).

1.3.4 PREY BASE

The size of Eurasian lynx home range can vary by a factor of ten within its distribution range (Herfindal et al. 2004). In southern Sweden, Eurasian lynx home range size declined with increased lynx density and for females, also with increased roe deer density (Herfindal et al. 2004, Aronsson 2016). Males, however, only increased the intensity of use of the most used parts of the home range with increased roe deer density (Aronsson 2016). In Poland, a population decline in roe deer and red deer (*Cervus elaphus*) resulted in increased home range sizes and thus decrease in Eurasian lynx density (Schmidt 2008). In Norway the home ranges also increased when prey population decreased, more rapidly so for males than for females (Herfindal et al. 2004). In a Swiss population though, no direct correlation between female home range size and habitat productivity could be given (Molinari-Jobin et al. 2007), but both sexes have been noted to reduce home range sizes with increased density (Pesenti & Zimmermann 2013). Vashon et al. (2008) suggest that for Canada lynx, female home range size indicates habitat quality, prey abundance and age structure in the population, with smaller ranges indicating better quality habitats and older individuals.

Lynx density in Finland varies naturally across the country (Luke 2019). In areas where small *cervids* are less numerous or absent, the lynx mainly preys on *lagomorphs*, mountain hare and European hare (Pulliainen et al. 1995).

1.3.5 HOME RANGE OVERLAP

In the *Lynx* genus, home ranges can be partly overlapping for both sexes. The extent of home range overlap varies between lynx species, sexes and study areas. The methods estimating the overlap differ, and while some studies use the entire home range for their calculations, others have concentrated on the core area, making sound comparisons difficult. The reason for the variance in the degree of overlap may be affected by density of prey and conspecifics, relatedness and sampling intensity (Kapfer 2014, Aronsson 2017).

For bobcats, both inter- and intrasexual home range overlap seemed flexible and based on local conditions such as prey abundance (Cochrane et al. 2006, Riley 2006, Young et al. 2019). Female home ranges were exclusive in the north of its distribution range (Lovallo and Anderson 1996) but not in the

south (Chamberlain and Leopold 2001, Cochrane et al. 2006, Riley 2006, Young et al. 2019), and male home ranges were highly overlapping in the north (Lovallo and Anderson 1996) but amount of overlap depended on habitat type in the south (Riley 2006, Young et al. 2019). Although core areas were typically more exclusive than the rest of the home range, some core area overlap was documented between females, and females and males (Chamberlain and Leopold 2001, Cochrane et al. 2006).

For Canada lynx, home range overlap varies greatly between study areas. In the geographic core of the population, overlap has been detected (Poole 1995), but not in the periphery when there is moderate prey abundance (Vashon et al. 2008). Core area overlap was substantially lower within males than females or between sexes (Poole 1995).

Results on Iberian lynx from a saturated population showed that home range overlap was higher between females than males but was overall quite low with exclusive core areas (Ferreras et al. 1997). Since the study was conducted, the species now has reproducing populations elsewhere, but no new data on home range arrangements in those populations have yet been published.

The Eurasian lynx seems to have some intra-sexual overlap but this differs between study areas and depends on whether the overlap is between males or females (Breitenmoser-Würsten et al. 2007, Schmidt et al. 1997, Aronsson 2020). In the Jura Mountains, males had slightly higher intra-sexual overlap than females, and the core areas remained relatively exclusive for both (Breitenmoser-Würsten et al. 2007). In Poland, however, overlap between males was high (30 %) while there was hardly any overlap between females (6 %) (Schmidt et al. 1997). In Scandinavia, intrasexual overlap was dependent on prey stability and relatedness (Aronsson 2016, Aronsson et al. 2020). With a stable prey base, male overlap was higher than that of females (Aronsson 2020). With a fluctuating prey base, female overlap was extensive between mother-daughter pairs, but not between other females (Aronsson 2020). Knowledge of the relatedness of neighbouring lynx in other species is very scarce over all or remains anecdotal with only a few studied individuals.

1.3.6 DISPERSAL AND EXPANSION

Before the mid- 20th century, the Finnish lynx population was located predominantly near the eastern border, owing to the large predator removal in the preceding decades, and recolonized the country after a partial hunting ban in the late 1960's. The recolonization thus took place from east to west with a few individuals translocated to the west coast in the 1980's. As to date, the peak of the population size was reached in 2014 when there was estimated to be about 2740-2890 over 1-year-old lynx in Finland (Luke 2014). The population developed in a manner of a still expanding population. In the east, where the lynx had been established the longest, the population in 2010's was stable with mostly full home ranges and a maximum number of breeding

individuals and high emigration. In the west, immigration and the number of subadults and breeders were high, but the best home ranges were already taken. The rest of the country lay somewhere within this continuum (Holmala *unpublished*).

The ability and speed of a species to expand to new, or recolonize former patches depends on its dispersal patterns and are bound to the social structure (Jerina and Adamič 2008). If even a proportion of the recruits stay philopatric, that is on or close to their natal home range, the colonization slows down considerably, compared to all recruits dispersing out of their natal home range (Blundell et al. 2002, Jerina and Adamič 2008). Dispersal and philopatry thus play a key role in expansion, recolonization and connectivity between populations (Bowler and Benton 2005).

In solitary *Felids*, both male and female offspring tend to disperse from their natal home range, but females settle closer to their mothers (Holecamp and Sawdy 2019). It is thought that this spatial closeness of female relatives promotes kin-favouring behaviour (Holecamp and Sawdy 2019). Competition for food and other resources is intense, especially as population density increases, but may be alleviated by sharing some of those resources with close kin, increasing the inclusive fitness (Holecamp and Sawdy 2019, Waser and Jones 1983, Hamilton 1964).

Male-biased dispersal has been documented in the bobcat and Eurasian lynx, but not in the Canada lynx or Iberian lynx (Poole 1997, Campbell and Strobeck 2006, Ferreras et al 2004), although the studies on Iberian lynx were conducted in a highly saturated population which may have influenced the result for the species. There is large variation in the dispersal distances for both sexes of the Eurasian lynx (Article I, Samelius et al 2012, Zimmermann et al 2005, Schmidt 1998). The dispersal distance affects the degree of spatial genetic structure between same-sex individuals (Banks and Peakall 2012). Emigration may also dilute the spatial genetic structure from genetically distinct populations (Biek et. al 2006, Zeyl et al 2009).

Sex-bias in dispersal may, on a local scale, be driven by inbreeding avoidance and differing needs of resources (Greenwood 1980, article III), but the cause of long-distance dispersal is likely expansion to new regions and is independent of sex (Perrin & Goudet 2001, Blundell et al. 2002, Ji et al 2009). When not compromised by fragmentation, dispersal and spatial organization can be expected to be driven by social, ecological and evolutionary constraints (Blundell et al. 2002, articles I-III).

2 OBJECTIVES AND HYPOTHESES

The objective of this thesis is to assess the role of dispersal and relatedness in the local population formation of Eurasian lynx in Finland. We hypothesized that:

Hypothesis I: Dispersal in the Eurasian lynx is male-biased. Males disperse earlier and travel further than females. In mammals, males generally disperse more often and earlier than females and their dispersal distances are longer than those of females (Greenwood 1980). The direction of sex-bias in dispersal is commonly explained by inbreeding avoidance and the polygynous mating system with male-male competition (Clark 1978, Dobson 1982).

Hypothesis II: Female Eurasian lynx form kin clusters that make up the backbone of the local population. In order to form these clusters, female offspring, or at least an adequate proportion of them, need to stay close to their natal home range and breed there, i.e. stay philopatric. The hypothesis derives from kin selection, which is an evolutionary strategy in which resources are shared with individuals of (usually close) kin if the costs of doing so do not outweigh the benefits (Hamilton 1964). Sharing increases the sharer's inclusive fitness if it helps the related individual to reproduce, as the sharer's genes are passed on as well. Sharing for lynx can mean sharing a part of its home range with adult offspring or a sibling, and is more common between females in mammals in general (Greenwood 1980). Females will contrast the male behaviour, by displaying a significantly lower degree of dispersal and thus higher genetic differentiation, clustering, and relatedness in space than males.

Three studies were conducted to assess these objectives and answer the hypotheses.

Article I: Movement patterns and habitat selection during dispersal in Eurasian lynx. In this study based on telemetry, we examined dispersal characteristics of both male and female lynx in Finland. We hypothesized that males disperse further than females, dispersal takes place mostly at night and that dispersing lynx use their habitats non-randomly.

Article II: Genetic analyses indicate male dispersal and female philopatry in Eurasian lynx in Finland. In this study, we assessed family structuring between males or males and females to know if dispersal is sex-biased. We estimated the degree of sex-specific patterns in dispersal and spatial organization as well as genetic diversity indices across our study area.

Article III: Genetic evidence of female kin clusters in a continuous population of a solitary carnivore, the Eurasian lynx. In this study, we investigated the genetic relatedness and family structure of Eurasian lynx females in southern Finland. Based on the knowledge of many mammalian species including several large carnivores, we hypothesize that female Eurasian lynx form matrilinear clusters based on female relatedness.

3 MATERIALS AND METHODS

3.1 CATCHING EURASIAN LYNX

In the telemetry study in article I, lynx were live-trapped by Luke personnel and voluntary assisting hunters in baited wire or wooden box-traps. The traps monitored by Luke were specifically made for catching lynx. The main targets for some of the voluntary hunters' traps were smaller carnivores such as foxes or raccoon dogs, but also lynx were an occasional by-catch. The lynx were immobilized by using a combination of medetomidine and ketamine. The animals were weighed and sexed and many other measurements were taken. The lynx were fitted with GPS collars with a remote GSM download technology and a programmable drop-off system (manufactured by Vectronic Aerospace GmbH, Germany; Followit, Sweden and Lotek, UK) and plastic ear-tags (Dalton, UK). Only the lynx that weighed 10 kilograms or over, and of those only the individuals in good general health judged by muscle condition were collared to minimize matters concerning the animal's welfare. The animals were given an anti-sedative for a rapid recovery from immobilization, their awakening was monitored in the trap and finally they were released at the trapping location. The capture and immobilization protocols were approved by the Finnish Animal Ethics Committee (ELLA) and evaluated for the ethical requirements for research on wild animals. In addition, permits for wild animal capture were obtained from the Finnish Wildlife Agency and Finnish Ministry of Agriculture and Forestry, the landowners and hunting rights' owners according to Finnish legislation.

3.2 FROM TELEMETRY DATA TO GIS ANALYSES

In total, 22 juvenile (born on or the year before collaring) Eurasian lynx were caught totalling seven females and 15 males (article I). The GPS- collars located the animals every four hours. Dense tree cover or lay downs in rocky crevices or other hiding places influenced the number of successful locations for some lynx, but not to a degree that would have compromised the integrity of the study. The movements were analysed using ArcGIS 10.2. (Redlands, CA, USA, ArcGIS Desktop 2014). The dispersal started from the first location outside the natal Kernel home range (calculated with Hawth tools -extension) and ended at the last location before accumulated data imparting the post-dispersal home range. In cases where the lynx was already dispersing when it was collared, the first location of radio tracking was treated as the dispersal start. Similarly, the last location was treated as the end when no post-dispersal

Kernel home range could be defined. Details of the methods we used for the GIS analyses using the lynx location data are described in article I.

3.3 HABITAT ANALYSIS, LAND-USE; DESCRIPTION

The study area was determined by the extension of the movements of dispersing lynx and included the whole country south of the reindeer management area. The area was classified into nine land-use classes on the Finnish CORINE land cover (CLC 2012) database with a 20 m pixel size. We used the second most refined class of the three available robustness levels and some similar classes were combined (article I). We selected the habitat variables that were meaningful to lynx based on our intensive monitoring field work experience of collared lynx. As an example, fields, pastures and other agricultural land were merged into one class.

We obtained the habitat data for the point locations (i.e. the used habitat) by intersecting the habitat layer from the CORINE data with the point location layer using ArcGIS 10.2. (Redlands, CA, USA, ArcGIS Desktop 2014). To obtain the habitat that was available for the dispersing lynx, we chronologically combined the point locations into a line and buffered that with a two-kilometre radius, forming a 4-km wide zone. We chose this zone width based on the mean travel distance (ca. 500 m) in a 4-hour interval by radio-tracked lynx (Holmala unpublished). We then intersected the buffered zone from the CORINE data and compared the habitat composition of the point locations (used habitat) to the habitat composition of the buffered region (available habitat). We analysed further with a resource selection function designed for this study (article I), which interpreted as the probability of selecting a habitat if all habitats were equally available. We did this for both daytime and nighttime selection for time-of-day comparison. The selection probabilities decreased in certainty the less the habitat is available. Details of the model can be found in the Appendix of article I.

3.4 SAMPLING OF GENETIC MATERIALS

In articles II and III the data consisted of legally hunted lynx and of those that had died in traffic accidents or were found dead in the South and South-East regions of Finland (i.e. Uusimaa and Kaakkois-Suomi regions) during 2007 - 2015. The area was chosen based on adequate data and dimensions large enough to include average dispersal distances. The lynx carcasses were sent to Natural Resources Institute Finland (Luke) field station where they were handled, sexed and sampled. A DNA sample was collected from the femur

muscle, preserved in alcohol and frozen. Ages of the individuals were determined from cementum annuli analysis of tooth samples by the Matson Laboratory, Milltown, Montana (Matson, 1981). DNA was extracted from the tissue samples using the DNeasy Tissue kit (Qiagen) in the laboratory of Norwegian Institute of Bioeconomy Research, NIBIO—Svanhovd. Detailed DNA extraction methods are described in the articles II and III.

3.5 GENETIC ANALYSES: SUMMARY OF METHODS USED IN THESE STUDIES

We used bi-parentally inherited STR/microsatellite markers. In the offspring, these allele frequencies are equally randomized between males and females. We used 23 (article III) and 21 (article II) STR –markers; 20 of which were originally identified for the domestic cat (*Felis catus*; Menotti-Raymond et al., 1999), whereas the remaining three markers were developed for the Canada lynx (Lc106, Lc109, Lc110; Carmichael et al. 2000). Two markers identified for the domestic cat were left out in article II because of unfeasible allele scoring in that data set. Also, gender was identified using the markers on the zinc finger region on the X- and Y-chromosome developed for felids (Pilgrim, McKelvey, Riddle, & Schwartz, 2005). The markers were chosen based on their use in several Eurasian lynx population genetic studies in northern Europe (e.g., Hellborg et al., 2002; Ruenesset al., 2003; Schmidt et al. 2009; Ratkiewicz et al., 2014; Rueness et al. 2014).

We checked whether the alleles under study were in Hardy-Weinberg equilibrium and calculated the expected and observed heterozygosity to understand the level of genetic variability, mainly to assess the level of inbreeding in our study population. This knowledge served as a good base for further genetic tests.

We applied STRUCTURE, a Bayesian clustering algorithm (Pritchard et al. 2000, Falush et al. 2003) to assess genetic clustering of lynx overall and, in article II, separately for each sex. In article II we investigated whether the sexes show differing levels of genetic variation partitioning among and within STRUCTURE clusters by performing an AMOVA (Analyses of Molecular Variance, Excoffier et al. 1992). To evaluate the relationship between genetic and spatial distance among pairs of lynx, we performed a spatial autocorrelation analysis (Peakall and Smouse 2006, 2012). In article I we evaluated this between all female lynx pairs and in article II between male and female lynx separately for each sex. Last, we calculated the F-statistics, i.e. aberrations from the expected Hardy-Weinberg equilibrium (Goudet 1995, Favre et al. 1997, Goudet 2002) which include five genetic diversity estimators.

In article II, we re-ran all the analyses using a LOCPRIOR function, which incorporates spatial information as a prior in the model. With the LOCPRIOR, the clustering algorithm assumes that the sampling location affects the

probability of an individual to get assigned to a cluster. Using the LOCPRIOR decreases the potential of finding false population structuring. We pooled the individuals into three regions; western, middle, and eastern part based on their sampling location, each containing a third of the samples.

4 MAIN RESULTS AND DISCUSSION

In our telemetry study, we could not distinguish female dispersal duration, distances, route lengths or linearity of the route from those of males, although the small number of females and the huge variation in all dispersal proxies probably influenced the results. Article I tells the story of those Eurasian lynx that do disperse. According to a telemetry study in Sweden, approximately two in three females disperse out of their natal home range, while the rest stay philopatric, close to their natal home range (Samelius et al. 2012). Using genetics (article III) we found that 65 % of the females had a close relative close-by, suggesting a higher degree of philopatry in the lynx in our study. Interestingly, some lynx established a home range close to their natal one, albeit travelling a great distance in between. Dispersal distance as a straight line from the start to the end comprised only 20 % of the total dispersal route. While dispersing, the lynx selected their habitat non-randomly just like resident lynx do (Podgórski et al. 2008; Filla et al. 2017; Gehr et al. 2017), using forest-covered habitats that provided ample hiding places and possibilities for catching prey. During daylight hours lynx were more discerning in their habitat selection, while most of the traveling took place at night, reflecting the crepuscular and nocturnal activity of the lynx. (Heurich et al. 2014).

The available landscape affects where lynx can settle. In many European populations, lynx habitat is small, fragmented and separated from each other by human-made barriers such as big roads and housing (Ratkiewicz et al. 2014, Schmidt et al. 2016). These patches are often saturated and the lynx population inbred, as dispersal is frustrated (Ratkiewicz et al. 2014, Krojerová-Prokešová et al. 2018). The available prey abundance and for example denning places set the limits on where the dispersers can settle, the home range sizes, their possible overlap and therefore the density into which the population has the potential to grow (Herfindal et al. 2005, Aronsson et al. 2017). In Scandinavia, there were noticeable differences in female home range size and overlap between home ranges situated in the sparse tree covered fjells with migratory prey (semi-domestic reindeer), and those in the boreal zone with year-round prey (roe deer) (Linnell et al. 2001, Herfindal et al. 2005, Aronsson 2020). Female home ranges decreased with lynx density and stable prey occurrence, but prey stability exhibited an inverse relationship with home range overlap (Aronsson 2020). For males, however, prey abundance did not have an effect on the home range size when also lynx density was accounted for (Aronsson 2016).

When female Eurasian lynx remain close to their natal home range, males need to disperse randomly further afield to avoid inbreeding (Greenwood 1980, Liberg and von Schantz 1985) and male-male competition (Sandell

1989). Thought to derive from the polygynous mating system and mammalian reproductive biology (Greenwood 1980), this may result in genetic clustering of female relatives but not of males (Kamler et al. 2013, Bailey 1993). Our study revealed male-biased dispersal (article II) and ensuing clustering of female relatives (articles II and III), but the telemetry data (article I) proves that the sex-bias is not complete. In a Polish study, females seemed to stay genetically, but not spatially, more philopatric than males, as related females were placed as randomly as any other lynx in the study area (Schmidt et al. 2016). Both in the Polish population and one in the Carpathian Mountains, two lineages of family groups dominated the areas, which in these rather isolated populations, increases the chances of inbreeding (Schmidt et al. 2016, Krojerová-Prokešová et al. 2018). In the Polish study, floaters that hung about for a vacant range were perceived to keep the population gene pool richer (Schmidt et al. 2016). Such floaters, predominantly males, were also found in the Carpathian study, but they did not seem to get chances to breed (Krojerová-Prokešová et al. 2018). In our study, the female genetic clusters highly overlapped in space, and about one third of the females did not belong to the clusters (articles II, III). We cannot know whether the females that remain in their natal local population have actually also dispersed as did some in our telemetry study (article I), and whether those who did, returned by chance or knowingly. This is, however, the first genetic evidence of local population structure and sex-bias in dispersal in Eurasian lynx, in which habitat fragmentation does not restrict dispersal and thus gene flow, so I conclude that the results we got essentially reflect the behaviour of a naturally working population.

The female clusters overlapped in space but were genetically well distinguishable (article III). The distance between closely related females was significantly shorter than between females overall (article III). Mothers and daughters were spatially closest, on average only 35 km apart, while the distance between sisters was 53 km, compared to the 223 km between females overall (article III). In Sweden, only the home ranges of mothers and daughters overlapped significantly in space, while full sisters did not (Aronsson et al. 2020).

The clustering is a phenomenon that takes several generations to evolve and requires its members to stay alive and succeed in raising female offspring that also stay close and breed. The role of the males is less imperative. The consecutive generations may be fathered by different males, as half of the genome is nevertheless preserved from the mother's side. It is known that a male that has a genetically distant enough genotype may break the clustering phenomenon on the genetic level (Goudet et al. 2002, Biek et al 2006). However, the real cluster-forming driver is female behaviour in the form of kin selection (article III). There were a few cases in our study (article III) where sisters were assigned to clusters differently (one assigned into a cluster, the other one not), indicating different fathers. A continuous turnover of either sex may also break the clustering process (Goudet et al. 2002, Biek et al. 2006).

We did not count the distances for males since they were randomly distributed over the study area. Lynx in adjacent home ranges are obviously aware of each other, and based on field work experience, may also meet. However, as kin clusters are established over several generations, it is not possible that each lynx has known all its relatives around. There can be individuals that are more central in the local population, either in space or in behaviour (Hackländer and Arnold 2012). It is also known that some females produce more viable offspring than others (Ribble 1992, Wauters and Dhondt 1995, Gompper et al. 1997, Kelly 2001) and that this trait is heritable (Kelly 2001). In the female clusters, we could distinguish an east-to-west gradient, representing the clusters in themselves, but perhaps also the recent recolonization history which took direction in a similar manner. When the lynx recolonized the country, it is possible that because of having a head start in reproduction, these females played a vital role in the clustering phenomenon. In a similar, still expanding population of Brown bears in Sweden, Støen et al. (2005) concluded that the loose formation of matrilineages detected in Brown bears may be due to differences in competitive abilities of the resident females in inhibiting non-kin females to establish home ranges in the area. This might work as a possible reason together with lack of success in raising philopatric daughters. Another explanation may come from bottom-up processes such as habitat quality, cub predation and possible competition among females that rises therein (Kelly 2001, Støen et al. 2005). We do not know if the maternal clusters in our studies started forming right away or only after a certain threshold in lynx density, but given their loose structure and the rather long time span in our study together with the fact that the lynx were hunted, i.e. taken out from the population, I doubt that density plays a critical role. There is evidence for inversely density-dependent dispersal behaviour for the species in Central Europe (Zimmermann et al. 2005, Breitenmoser-Würsten et al. 2007).

The division between something being local or regional are scale-dependent and can vary according to the research question (Yannic et al. 2012, Coulson et al. 1997). We defined local population as those lynx that live inside a maternal cluster defined by both genetic and spatial closeness (articles II, III). The clusters were rather loose, on average about 100 – 150 km in width in their core areas (articles II and III), built over our study's 15-year time span of female offspring settling close to their natal range. In contrast, our regional scale would need to encompass that of male dispersal distances, which, at 65.5 km on average (article I), were nearly double the distance of the locations of mother-daughter pairs (article III). The hypothesis of sex-biased dispersal in mammals leans on females as the backbones of the population, because they stay more or less in their natal area while males disperse out of it (Greenwood 1980). Therefore, the females' impact on forming the local population is higher than that of males.

4.1 HUNTING OF EURASIAN LYNX IN FINLAND

In articles II and III, our data consisted mainly of hunted Eurasian lynx. The recolonized, growing lynx population was met with an increase in hunting quotas, which in the 2010's cut circa 20 % of the population yearly (Luke 2019). Lynx are mainly hunted using scent-trailing hounds with GPS-collars that drive the lynx to the shooters. The hunting is described to be non-selective, in which the hunting bag represents the age and sex structure of that in the population, although females with cubs are principally protected. The hunting bag of lynx in Finland typically includes both sexes with males slightly in the majority (Luke 2019).

There are examples of the effect of hunting on the social organization and dispersal in North American cougar and African leopard populations. The difference in the reproductive strategy between the lynx and these bigger felines lies in infanticide, which the lynx are not known to have, in part because of their synchronized reproduction period in early spring (Ebensperger 1998). In a cougar population, heavy hunting seized male emigration because of high mortality of dispersers (Newby et al. 2013). Female cougars emigrated less than in an undisturbed population, probably because the high turnover rate provided chances of philopatry (Newby et al. 2013). In a heavily hunted population of African leopards, male dispersal ceased, and more male offspring remained in their natal population (Naude et al. 2020, Fattebert et al. 2015) as trophy hunting of mature males increased the male turnover rate (Fattebert et al. 2015). The high turnover rate of males also increased the rate of infanticidal events (Fattebert et al. 2015) and lowered the mating success and increased time between litters, which may translate as lower lifetime reproductive success (Balme et al. 2009). The synchronous mating period and absence of infanticide in the Eurasian lynx does not imply quite the same outcome. Female preference for resident males could potentially lead to lowered mating success if hunting removes such individuals during the mating season, which partly coincides with the hunting season.

Adult mortality in large carnivores affects the rate of population increase the most (Sæther et al. 1998). Intensive hunting can start a fluctuation in the population, for which it becomes less predictable from a management point of view (Andrén et al. 2006, Fryxell et al. 2010, Sæther et al 2010, Newby 2013, Teichman 2016). In the Finnish system, if the number of family groups in a given area varies greatly between years, the reduced predictability increases the need for additional information from both the management and stakeholder point of views, both of which increase the burden on volunteer large carnivore contact persons. Without the detailed information on local circumstances, it becomes undeniably difficult to lean on science when addressing derogation licences.

Disturbing the age structure in a species with high adult survival such as Eurasian lynx (when not hunted) (Andrén et al 2006), the generation time and

thus return time to a stable stage on the population level is likely large (Sæther et al 2010). Males are more likely to be hunted either for their trophy value or because of their behaviour (Bunnefeld et al. 2006, Newby 2013). Increased hunting was not responded to by increasing recruitment, birth rates or survival in a population of bobcats (Knick 1990). A harvest level that reached just above 20 % of the population after reproduction (i.e. in the autumn) also resulted in large changes in the population, as home ranges were vacated faster than they were filled because of lack of dispersing young (Knick 1990). Home ranges are predominantly reoccupied by a follower of the same sex (Benson et al. 2004). While not intensively studied in the Eurasian lynx, the outcomes seem to agree with those of the bobcat population. In a Eurasian lynx population in the Jura mountains, the home ranges of two poached males were filled only three and five years later (Breitenmoser-Würsten et al. 2007), reflecting perhaps the outcome of frustrated dispersals in the fragmented Central European populations.

A high turnover rate of males, i.e. a low degree of generational overlap, may cause a decrease in genetic structuring in the opposite sex (Blyton et al 2015). This may not seem solely a negative thing. In a model by Chesser (1990), the genetic variability among lineages within a population was smallest when a polygynous male bred within a lineage of philopatric females (Chesser 1990). The proportion of the variance depended on the lineage size and the number of breeding males in each lineage (Chesser 1990). For example in a hunted population of red fox (Vulpes vulpes) in Europe, genetic variability was higher than in a non-hunted population (Frati et al. 2000). Hunting could mimic the predation pressure under which foxes evolved by increased turnover and immigration of new alleles into the population (Frati et al. 2000). However, in a leopard population, high male turnover rate resulted in opportunistic male philopatry, male kin clusters and significant inbreeding compared to a population with a balanced social organization (Naude et al. 2020). For a subadult male, the opportunity to avoid costly dispersal may well exceed the pressure to avoid breeding with kin, especially in the absence of conflict with resident males.

However, many of the population–level consequences of alterations in genetic structuring are still not understood. Adult males have valuable genes as they have survived dispersal and succeeded in holding a territory. Selection acts to fix the good genes, decreasing variance in genetic quality (Neff and Pitcher 2005). The effect of high turnover rate may thus be evident in the genetic quality of the breeders, most profoundly for males, and not be easily recognizable (Harris et al. 2002).

Considering that we found matrilineages that encompassed many generations, we are left with the fact that these members in the matrilineages were taken out of the population. Removing such a big proportion of genetic material that a matrilineage holds, from the population, can eventually have severe effects on the genetic diversity of a species (Kelly 2001). Matrilineages can be lost rather quickly in natural populations because of ecological or

behavioural processes such as mating ability or territory quality, either randomly or through a selection process, but hunting will undoubtedly exacerbate the losses (Gompper et al. 1997). The lynx population has proved to be capable of rebounding when hunting pressure decreases, but there are several examples in Finland where a home range formerly occupied by a breeding female, does not get filled for years (Holmala unpublished). While demographic effects can be easily recognizable, genetic effects may take generations to become apparent (Harris et al. 2002). For now, the Finnish Eurasian lynx population is continuous with that of Russia and holds no dispersal barriers within Finland. However, the Scandinavian population is a good example of withered gene flow in an apparent continuous habitat. Gene flow between Finland or Russia and the Scandinavian lynx population has practically ceased because of the barrier caused by the reindeer husbandry area on both sides of the Swedish, Norwegian and Finnish borders (Hellborg et al. 2002, Rueness et al. 2003). Also, the Finnish wolf population has very limited gene flow with the Russian population nowadays (Aspi et al. 2009). Intensive forestry fragments habitat within home ranges, i.e. on a small scale, and may force lynx to shift or move which may affect the social organization in the local population (Frank 2017). On top of the effects that habitat fragmentation and hunting may have on a population, other species may also affect the species of interest either directly or in interaction with others (Newby 2013). For example, in a cougar population, the reintroduction of wolves seemed to affect habitat use in cougars and promote emigration from the area (Newby 2013).

4.2 CONCLUSIONS: LOCAL POPULATION DYNAMICS FOR THE EURASIAN LYNX

The description of a local population is inevitably arbitrary as neither our study area, nor the maternal clusters had natural borders, and the female assemblages were highly overlapping in space (articles II and III). Eurasian lynx population dynamics take place on different levels of scale. Local scale geography, prey populations and their abundance together with human land use and hunting direct lynx settlement, the overlap of home ranges and lynx population dynamics (Aronsson et al. 2020, Sæther et al. 2010). In a naturally working lynx population like that in Southern Finland, the importance of the forces that affect what the local population consists of, work differently for female and male lynx.

For females, relatedness to adjacent home range residents facilitates settling (Krojerová-Prokešová et al. 2018) but is nevertheless bound to available resources such as den sites and prey (Aronsson et al. 2020). A well-established cluster can bear random interruptions in its structure and

naturally includes unrelated individuals too (articles II, III). This is evident from our results; the matrilinear clusters were rather loose formations, judging by the individuals taken out from the population. If we knew the status of the remaining individuals, we could ascertain the texture of the cluster better. Hunting adult females disturbs the clustering pattern and may exacerbate the loss of matrilineages and genetic diversity (Gompper et al. 1997).

For males, relatedness is inversely important as they tend to separate themselves from close kin (article II). The apparent ultimate reason for this is to avoid inbreeding, which in the case of clustering female relatives, requires an adequate dispersal distance. The consequences of the loss of a resident male lynx are likely genetic and are harder to assess in a species with no infanticide, but the instability created in the population may have an effect on genetic quality and the birth rate in the following reproductive season. This is especially true if the male is hunted, as the hunting season for lynx in Finland takes place during or just before the peak reproductive season.

4.3 RECOMMENDATIONS FOR EURASIAN LYNX MANAGEMENT AND CONSERVATION IN FINLAND

The females' tendency to cluster with their female relatives means that the forming of matrilineages hold much of the genetic variance that has kept the Finnish lynx population healthy, together with those resident males that have survived dispersal and succeeded in establishing a home range. Lynx hunting has been assumed to be non-selective, in which the hunting bag represents the age and sex structure of that in the population. The high number of adult females in our data strongly suggests that this has not been the case. Hunting regimes that produce mortality patterns simulating those that occur in nature, and which aim to maintain the demographic structure of the species' breeding arrangements, will have fewer evolutionary consequences in the long term, compared to those that produce highly atypical mortality patterns (Harris et al. 2002). This suggests that the hunting bag should consist of mostly young lynx, as the natural adult survival rate for the Eurasian lynx is high (Andrén et al 2006).

Females with cubs are principally protected from the hunt, but the outcome is not surveyed by authorities. I suggest some pressure could be added from the authorities, as shooting females with dependent cubs is detrimental for the cubs themselves, but also involves a strong moral question, which legal protection should directly address. In my personal observation, female Eurasian lynx may leave their dependent young behind when they go hunting for prey into mid-January, i.e. in the middle of the hunting season, leaving

single tracks instead of those of a family group. This, together with an inadequate snow cover in the south of the country, creates circumstances where the hunters cannot ascertain their target well enough to avoid shooting a female with cubs.

Finland's Eurasian lynx population is viable. To keep it that way, and to be able to continue the hunting traditions also in the future, we should aim to preserve the reproducing lynx to raise the subsequent generation.

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ORIGINAL PAPER

Movement patterns and habitat selection during dispersal in Eurasian lynx

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Abstract

Natal dispersal affects the gene flow, distribution, dynamics and social structure of a population. In many solitary mammals, dispersal is often male-biased, while females may remain philopatric. For the Eurasian lynx (*Lynx lynx*), dispersal distances vary greatly and may be explained by sex or by habitat characteristics, such as fragmentation. Juvenile lynx habitat selection during dispersal has not been studied before, but resident lynx select heterogeneous forested habitats and avoid human settlements and infrastructure through distinct temporal activity patterns. We studied movement patterns and habitat selection during the dispersal of 22 Eurasian lynx (7 females, 15 males) in Finland. We found no differences between the sexes in the onset age, duration, distance, route or route linearity. Dispersal took place mostly during the evening and at night but also during the day. Of the four most used habitats, the mixed forests and transitional woodlands were used more frequently than would be expected according to their availability, and conifer forests and fields less than would be expected. Housing and other human infrastructure, including roads, were the least frequently used categories. There was a highly significant difference between the used and available habitats in general. The lynx selected the habitat more carefully during the day than during the night.

Keywords Lynx (*Lynx lynx*) · Dispersal · Movement patterns · Habitat selection · Resource selection function

Introduction

Natal dispersal, which is the movement from a natal site to a breeding site, affects the gene flow within a population as well as the distribution, population dynamics and social structure (Bowler and Benton 2005). Understanding how and why individuals disperse is essential for population management and for predicting the persistence and future distribution of a species (Cote et al. 2010). In most mammal species, males disperse further than females (Greenwood 1980; Dobson 1982). This is thought to be one of the mechanisms to avoid inbreeding (Greenwood 1980; Liberg and von Schantz 1985) and to decrease male-male competition (Sandell 1989). In many solitary mammals, females may remain philopatric. Philopatry can be considered in different scales. Sometimes, philopatry is described as movement no further than ten home ranges away from the original site (Shields 1987) and sometimes simply as staying in the natal range or in a portion of it, and dispersal as movement out of the natal home range (Greenwood 1980; Waser and Jones 1983).

In the *Lynx* genus, male-biased dispersal has been documented in bobcats (*Lynx rufus*) (Janečka et al. 2006) but according to a molecular genetic study, not in Canada lynx (*Lynx canadensis*), possibly because of its cyclic population dynamics (Campbell and Strobeck 2006; Breitenmoser et al. 1993). A male bias has been reported based on radio-telemetry studies of Eurasian lynx (*Lynx lynx*) in Scandinavia (Samelius et al. 2012) but not in Central Europe (Zimmermann et al. 2005, 2007). In Eurasian lynx, natal dispersal usually occurs when new kittens are born between mid-May and mid-June;

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however, the age at onset varies from 8 to 24 months (Breitenmoser et al. 1993; Schmidt 1998; Zimmermann et al. 2005; Samelius et al. 2012).

In Central Europe, Eurasian lynx dispersal distances are substantially shorter than those in Scandinavia, although individual variation is large. In Central Europe, males dispersed 4.5–129 km, compared to 32–428 km in Scandinavia (Breitenmoser et al. 1993; Schmidt 1998; Zimmermann et al. 2005; Samelius et al. 2012). Females in Central Europe dispersed 2–81 km compared to 3–215 km in Scandinavia (Samelius et al. 2012). In Scandinavia, one third of female offspring did not disperse but established an overlapping home range with their natal home range (Samelius et al. 2012). Based on genetic studies, Eurasian lynx females in Finland seem to form kin clusters (Holmala et al. 2018), which are overlapping, genetically distinguishable structures suggesting at least partial philopatry.

In Central Europe, the availability of suitable habitats and the arrangement of vacant home ranges steer the direction and length of dispersal by Eurasian lynx (Schmidt 1998). Habitat selection by dispersing Eurasian lynx has not been previously studied, but studies on cougars (*Puma concolor*) and African leopards (*Panthera pardus*) reveal that dispersers select habitats non-randomly, as do resident adults in their home ranges (Zeller et al. 2014; Fattebert et al. 2015). However, dispersers are known to use a wider array of habitats and even traverse habitats thought to be impermeable for the species (Gastón et al. 2016; Vanbianchi et al. 2017). The time of day, prey availability and hunting conditions are known to influence lynx home range use (Podgórski et al. 2008; Filla et al. 2017; Gehr et al. 2017). Additionally, day resting sites are selected in areas away from all human infrastructure (Filla et al. 2017; Gehr et al. 2017; Signer et al. 2019). Lynx have a distinct circadian activity pattern (Heurich et al. 2014). The overall daily activity level in lynx is not influenced by the daylight duration, but the activity pattern is (Heurich et al. 2014). In areas with a complete day-night cycle, such as in Finland during spring and autumn, lynx activity is lowest during the day and highest at night, peaking at crepuscular times (Heurich et al. 2014). However, the bimodal differences and crepuscular peaks in activity gradually smoothen for lynx in the higher polar latitudes, and dispersing subadults are more active during the day than adults (Heurich et al. 2014).

It is unclear whether the travel between habitats is based on the nearest visible area, with route planning occurring during movement. To evaluate the dispersal and its link to habitats, we analysed the dispersal movement parameters of 22 Eurasian lynx in Finland. We hypothesised that (1) males disperse further than females, (2) dispersal movement takes place mostly at night and (3) dispersing lynx use their habitats non-randomly. No barriers to the dispersal of Eurasian lynx are known in Finland, although large roads and lakes may direct the route. Therefore, the habitat selection by dispersing lynx can be studied without specific corridors channelling selection decisions.

Material and methods

Study area

The study was conducted in southern and central Finland (61°N, 25°E; Fig. 1), encompassing about two thirds of the country (approximately 237,000 km^2). We defined the study area by the national borders except in the north, where we set the limit to include the area used by the northernmost dispersing lynx. This was because we do not collar lynx in the reindeer herding area. The area was located in the boreal zone, which comprises a mosaic of agricultural land and coniferous (spruce *Picea abies*, pine *Pinus sylvestris*) and deciduous commercial forests (silver birch *Betula pendula*, downy birch *Betula pubescens*, black alder *Alnus glutinosa*, grey alder *Alnus incana*, aspen *Populus tremula*, bird cherry *Prunus padus* and rowan *Sorbus aucuparia*). Small settlements and several small- and medium-sized lakes are situated within the area. The mean annual temperature in the middle of the study area is 4.2 °C, ranging from − 6.5 °C in January to 16.6 °C in July. The ground is covered with snow for approximately 6 months of a year. The human population density is from 3.9 people/km^2 in the north to 170.4 people/km^2 in the most crowded areas in the south.

GPS-GSM radio-tracking

A total of 22 juvenile Eurasian lynx (15 males, 7 females) were captured and radio-tracked during 2009–2017. The lynx were captured mainly with wire or wooden baited box traps. The lynx were immobilised by using a combination of medetomidine and ketamine. The animals were weighed, sexed and fitted with GPS collars with remote GSM download technology and a programmable drop-off system (manufactured by Vectronic Aerospace GmbH, Germany; Followit, Sweden and Lotek, UK, respectively) and plastic ear-tags (Dalton, UK). Only the lynx weighing 10 kg or over and in good general health were fitted with radio-collars, the most typical collars used in our study representing approximately 3–4% (or less) of the individual's weight. This was to ensure that mostly "normally" behaving individuals are tagged and to minimise ethical issues concerning the animal's welfare. The age was determined by the lynx's weight, body proportions, tooth wear and general appearance. The animals were released at the trapping location. The research plan and immobilization protocol were approved by the Finnish Animal Ethics Committee (ELLA). In addition, permits for capture were obtained from the Finnish Wildlife Agency and Finnish Ministry of Agriculture and Forestry. The collars

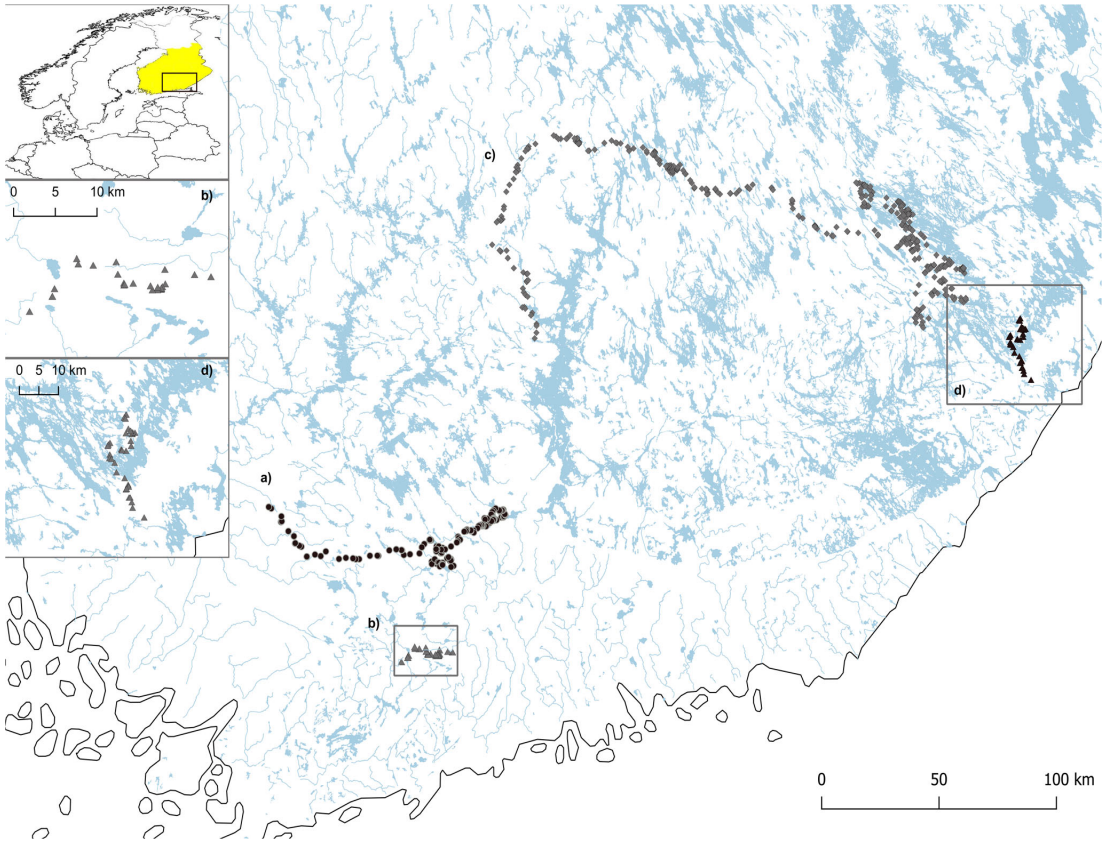

Fig. 1 The dispersal of Eurasian lynx was studied in an area that encompassed about two thirds of Finland. In the close-ups: examples of long-distance (**a**, **c**) and short (**b**, **d**) dispersal by female (**a**, **b**) and male (**c**, **d**) Eurasian lynx. We could not present all 22 dispersals on the same map because of overlap

transmitted the locations of the animals typically at 4-h intervals for a total of six times per day.

Dispersal onset, distance, route and linearity

For this study, we defined *dispersal* as the movement from a natal site to a potential breeding site and *philopatry* as staying in or in close proximity of the natal home range. We defined close proximity as a distance of a maximum of one home range radius away from the natal home range. For this description, an imaginary circular home range with 10 km radius was used, which is also the model home range used for lynx population monitoring in Finland, modified from Scandinavian monitoring method (Linnell et al. 2007; Luke 2008-2018). Thus, if the dispersal distance exceeded 20 km, an individual was considered to have dispersed.

The existence of a potential home range (either natal or post-dispersal) was estimated visually in a GIS program and was based on the spatial arrangement of the chronological point locations. If locations clustered together constantly creating a stable pattern, the individual was categorised having a clear home range. Varying time periods were used; however, the data accumulation needed to be enough to confirm a pattern in the movement behaviour and allow a calculation of a Kernel home range. For a few individuals, the natal home range site was known based on field monitoring of the marked individual moving together with its parent (known pairs of parent-offspring) before leaving the area. Typically, the take-off was a rather straight forward movement out of the natal home range, with no return. The end of the dispersal was estimated to have been reached when the accumulation of the point locations formed a stable pattern in a specific area for over varying period of time, but so that a Kernel home range could be calculated (Table 1).

For determining the travelled distance when a natal or post-dispersal home range could not be calculated based on telemetry data, the first point location outside or on the exact border of a calculated minimum convex polygon (MCP 100%) was considered the start point of the dispersal. The start was known for 10 individuals (Table 1) and not known for 12 individuals, for which the first location of radio tracking was used. The endpoint of the dispersal was considered as the last

Table 1 The timing, minimum durations and distances of the dispersal events and the number of successful point locations (fixes) for the radio-tracked Eurasian lynx ($N_{males} = 15$; $N_{females} = 7$). x = data achieved during this study, (x) = data retrieved after the study, NA = data not available

ID	Sex	Dispersal	Duration days	Fixes	Start month	Known start	Known end	Dispersal distance km	Dispersal route km
F1	F	30/12/2008–10/2/2009	43	169	December	NA	x	75.47	220.3
F2	F	13/4/2013–30/6/2013	109	435	April	NA	(x)	16.28	386.0
F3	F	11/4/2013–6/6/2013	57	243	April	NA	x	101.20	209.8
F4	F	17/5/2008–23/5/2008	7	39	May	x	x	22.10	32.7
F5	F	24/3/2012–18/5/2012	56	265	March	NA	NA	3.85	72.4
F6	F	19/9/2014–23/12/2014	96	546	September	NA	NA	32.88	447.4
F7	F	1/4/2010–3/1/2011	278	1268	April	x	NA	30.28	1177.8
M1	M	8/4/2010–26/12/2010	263	1043	April	NA	NA	25.39	1067.8
M2	M	25/4/2012–9/9/2012	138	740	April	NA	x	51.66	490.7
M3	M	16/4/2012–10/2/2013	301	1575	April	NA	NA	124.98	1277.6
M4	M	29/8/2011–9/9/2011	11	32	August	NA	(x)	24.02	43.0
M5	M	16/4/2012–25/5/2012	40	192	April	NA	x	45.52	138.4
M6	M	24/7/2015–5/1/2016	166	916	July	x	NA	150.82	778.9
M7	M	19/4/2017–3/9/2017	137	781	April	x	NA	31.55	676.4
M8	M	10/4/2011–20/5/2011	41	140	April	x	x	40.23	150.1
M9	M	2/7/2011–8/12/2011	160	480	July	x	x	52.30	652.2
M10	M	21/4/2011–6/7/2011	77	410	April	x	(x)	91.87	159.9
M11	M	13/4/2014–9/9/2014	150	677	April	x	(x)	30.48	591.1
M12	M	11/7/2009–1/12/2009	144	635	July	x	x	170.98	782.7
M13	M	15/10/2010–2/1/2011	78	481	October	x	x	83.66	502.8
M14	M	24/9/2014–10/12/2014	77	408	September	NA	x	27.04	407.2
M15	M	13/2/2010–24/4/2010	70	227	February	NA	(x)	15.65	158.0

point location outside or on the exact border of a calculated post-dispersal MCP 100% home range for 10 individuals, and for seven individuals, the last location from the radio-tracking data. Additionally, five lynx (one female and four males) with unknown dispersal endpoints were later encountered as adult hunted specimens with known death locations, thus giving the areal location of the end point of the dispersal. Three of these were encountered on their earlier dispersal routes, suggesting that some of their dispersal routes may represent a part of their post-dispersal home range. For four individuals, neither the natal nor the post-dispersal home ranges could be verified from the point location data or other information.

The dispersal distance, or the A to B distance, was calculated as the straight-line distance from the first to the last dispersal location. The route distance was calculated as the distance between all locations in the chronological order. Because of the unknown dispersal start and/or endpoints for some of the animals, all calculated distances were effectively

Table 2 Land-use classes used in the study, the classes included in them and their availabilities in the study area based on the Finnish CORINE Land Cover (2012)

Land-use class	Included classes	Availability (%)
Infrastructure	Sports areas industrial and traffic areas roads	0.7
Peatland	Forested and open bogs	0.8
Broad-leaved forest	Deciduous trees as dominating species	0.9
Housing	Permanent housing and holiday apartments	1.7
Transitional woodland with sparse tree cover areas	Forest land with sparse tree cover density < 30% including sapling stands clear cuts and rock land	7.00
Water bodies	Wetlands and open water bodies	11.3
Fields	Fields pastures and unused farmland	18.4
Mixed forest	Conifers and deciduous trees	25.8
Conifer forest	Conifers as dominating species	33.4

the minimum distances except for the five individuals for which all data could be collected. The linearity of the dispersal route was calculated with a *distance to route*, *or linearity index* (Bell and Kramer 1979), which was simply the proportion of the distance length to the total route length as a percentage. The index revealed how linear the dispersal route was. A high index value implies a relatively straight path, whereas a small value indicates that the animal meandered considerably (Bell and Kramer 1979; Spencer et al. 1990). For the five individuals who were found to have been hunted, the dispersal distance was measured to both the end of the collar function (A to B) and to the death location (A to C).

Habitat data

We classified the study area's landscape into nine different land-use classes based on the Finnish CORINE Land Cover (CLC 2012) database, a standardised land-cover classification system in the European Union, which has a 20-m pixel size (Finnish Environment Institute 2012). There are 51 land-use classes in CORINE that are present in Finland, with four robustness or resolution levels. We used level 3, which was the second most refined class, and combined some similar classes together (for example, several human infrastructure classes were combined). We used our field work experience to determine the meaningful land-use classes for lynx. The used classes and the classes that were merged into those in level 3 as well as their availabilities in the study area are listed in Table 2.

We studied habitat use and habitat selection by intersecting point locations with habitat map and by comparing the habitat composition of the dispersal point locations (used) to those of the buffered travel route (available). We combined the chronological point locations into a line that we buffered with a two-kilometre radius, forming a 4-km-wide zone which was then overlaid with the habitat map. We chose the buffer width based on the mean distance travelled per 4-h interval (approximately 500 m) of radio-tracked lynx (K. Holmala, *unpublished*). In theory, an individual could start from the known point and travel 2 km within the 4-h interval. However, in order for this same individual to be in the next known point at the 4th hour, an animal could actually travel only 1 km away from its trajectory and then back. Thus, we considered 2-km buffer to represent adequate potential area available for an individual. All GIS analyses were performed using ArcGIS 10.2. (Redlands, CA, USA) (ArcGIS Desktop 2014).

Statistical analysis

For the analysis of diurnal variation in the travel, the mean dispersal speed of each lynx was computed separately for each 4-h interval. The statistical significance of the differences between the time intervals in the mean speed over all lynx was evaluated by fitting an ANOVA model with individual-level random effects. The pairwise comparisons between the time intervals were obtained with Tukey's post hoc test (see Hothorn et al. 2008).

The ratios of habitat use over their availabilities were analysed with resource selection functions that followed the approach described by Aarts et al. (2008). The details are provided in Appendix .

We also tested how well the Manly-Chesson selection index, which is the habitat use divided by the habitat availability (Manly et al. 1972; Chesson 1978), described the lynx habitat selection. This index value was < 1 if the habitat was avoided and > 1 if the habitat was selected.

Because the dispersal variables were not normally distributed, we used the Mann-Whitney *U* test to examine the differences in the dispersal durations, distance (A to B distance) routes and distance to route indexes and Spearman's rank order correlation for the dispersal durations, distances (A to B distance) and routes. We set the level of significance to 0.05 and used SYSTAT 13 and IBM SPSS Statistics 25. The analyses of the diurnal variation and resource selection functions were conducted in the R environment (R Core Team 2018) using the nlme package (Pinheiro et al. 2018) to fit the models with random effects and the multcomp package (Hothorn et al. 2008) to obtain the Tukey's tests.

Results

Dispersal age, onset, duration, distance, route and route linearity

The median age at the onset of lynx dispersal was 10 months, ranging from 7 to 16 months. Both sexes typically began to disperse in April, also when only individuals with a known dispersal start were considered. Some lynx were caught and marked while they were already dispersing. Hence, their dispersal duration, distance and route are to be considered minimum values. In general, the females travelled shorter distances according to the straight line dispersal (A to B distance), with an average of 37.8 km for females (SD = 35.61) and 65.5 km for males (SD = 48.63), but the differences between the sexes were not significant ($N_{females} = 7$, $N_{males} = 15$, Mann-Whitney $U = 34{,}000$, $p = 0.192$, $d.f. = 1$; Table 3). We also counted the distance from dispersal onset to the death place for the one female and three males that were later encountered as hunted individuals (A to C distance). This increased the female average dispersal distance to 42.32 km (SD = 34.42) and 67.86 km (SD = 49.68) for males but decreased the significance between sexes ($N_{females} = 7$, $N_{males} = 15$, $U = 70{,}000$, $p = 0.217$, $d.f. = 1$). For the total route length travelled during the whole dispersal period, no differences between the sexes could be distinguished (Mann-Whitney

Table 3 Movement characteristics of the dispersing radio-tracked Eurasian lynx. For the pooled[1] individuals ($N = 5$), comprehensive data has been obtained. For the pooled[2] ($N = 22$), which includes all studied individuals, the distances are effectively minimum values because of the shortage in data availability for most individuals

	Duration (days)	SD	Distance (km)	SD	Route (km)	SD	Route straightness index	SD
Females ($N = 7$)	92.3	88.55	37.8	35.61	363.6	383.4	0.20	0.26
Males ($N = 15$)	123.5	80.76	65.5	48.63	564.8	370.6	0.19	0.18
Pooled[1] (N = 5)	86	65.5	73.9	58.7	424.1	322.1	0.30	0.20
Pooled[2] ($N = 22$)	113.6	82.53	56.7	46.00	500.8	377.5	0.21	0.20

$U = 36{,}000$, $p = 0.245$, $d.f. = 1$, $\text{mean}_{\text{female}} = 363.6$ km; SD = 383.39; $\text{mean}_{\text{male}} = 564.8$ km; SD = 370.61). This was also true for the distance/route-percentage index describing the travel linearity (Mann-Whitney $U = 50{,}000$, $p = 0.860$, $d.f. = 1$) and dispersal duration ($\text{mean}_{\text{females}} = 92$ days, $\text{mean}_{\text{males}} = 124$ days, Mann-Whitney $U = 37{,}000$, $d.f. = 1$, $P = 0.274$). There were no statistically significant differences in any of the dispersal parameters between all individuals and those five individuals for which dispersal start and finish were known.

The observed dispersal speed was highest at 16:00–20:00 h and lowest at 08:00–12:00 (Table 4, Fig. 2). The speed was significantly higher in all 4-h intervals between 16:00 and 04:00 than in any of the other intervals between 04:00 and 16:00. The dispersal duration was highly correlated with the route ($r_s = 0.949$, $N = 22$, $p = 0.001$) but not with straight line dispersal distance ($r_s = 0.161$, $N = 22$, $p = 0.161$).

Habitat use and selection

When we compared the habitat use, the lynx used four habitats most frequently during dispersal: the mixed forests (42.9%), conifer forests (27.8%), fields and other agricultural lands (14.6%) and transitional woodlands (11.8%). Of these, the mixed forests and transitional woodlands were used more frequently than would be expected according to their availability, and conifer forests and fields less than would be expected according to their availability (Fig. 3). Additionally, broad-leaved forests were used more than would be projected with their availability, but all other habitats were used less than would be expected based on their availability. The use of forests or canopy-covered areas increased during the daytime, while that of fields decreased. During the night, fields were used more than would be anticipated according to their availability.

When looking at the resource selection functions, both the main effect of habitat and its interaction with the time of day were highly significant predictors of use-availability ratio. The transitional woodlands and mixed forests were always selected with significantly greater probability than those of the coniferous forests or water bodies (Table 5). In the daytime, all forests and transitional woodland were selected with significantly greater probability than were fields, but the difference decreased during the night. Estimates of the selection probabilities had great uncertainty for the broad-leaved forests, peatlands, housing and infrastructure because of their small areas among the available habitats (Fig. 4).

Also the other used index for habitat selection, the Manly-Chesson selection index showed similar results for most of the habitats but failed to describe use for the peatlands, which

Table 4 Distributions of individual-specific mean dispersal speeds (km/h) in each 4-h time interval. Means with the same lowercase letters are not significantly different

Time	Min	1st quartile	Median	3rd quartile	Max	Mean
00–04	0.06	0.21	0.22	0.25	0.34	0.22ab
04–08	0.04	0.07	0.10	0.13	0.39	0.11cd
08–12	0.01	0.04	0.06	0.07	0.20	0.06c
12–16	0.03	0.08	0.13	0.19	0.32	0.15ad
16–20	0.08	0.22	0.27	0.35	0.47	0.27b
20–24	0.11	0.24	0.28	0.30	1.11	0.30b

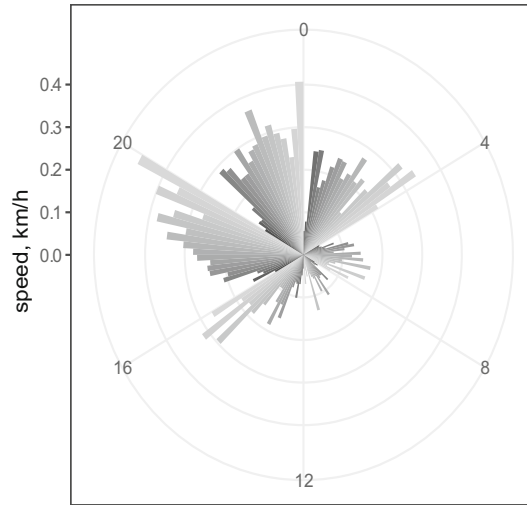

Fig. 2 Individual-specific mean dispersal speeds (km/h) of Eurasian lynx in each 4-h time interval describing the distribution of activity between time intervals and individuals. Same shade of grey level indicates the same individual. The individuals are in the same order within each 4-h interval. The order is determined by their overall mean dispersal speed

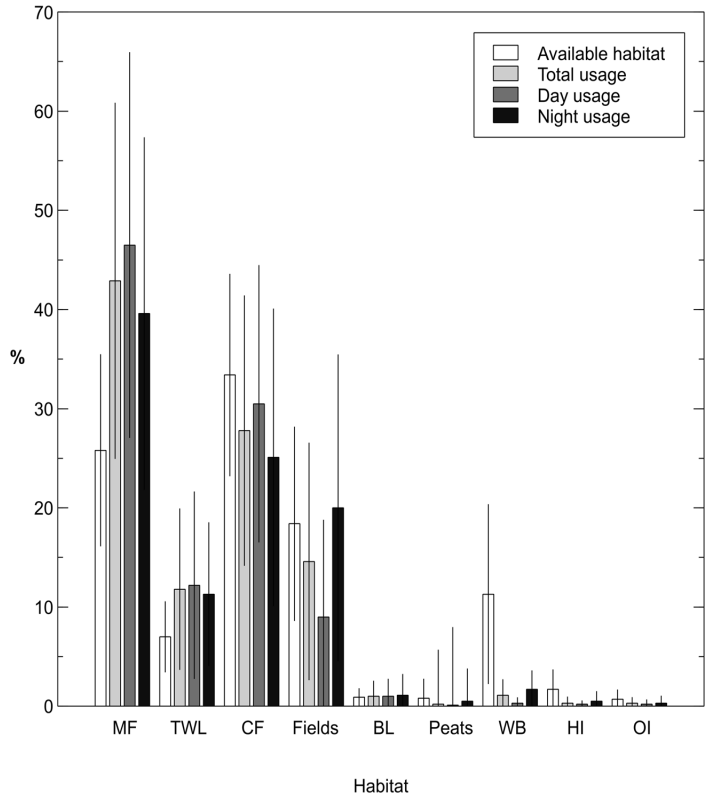

Fig. 3 Habitat use of Eurasian lynx ($N = 22$) during dispersal and the composition of available habitat types on their dispersal routes in the landscape. The habitat use was determined according to the point location data and the CORINE 2012 Land Use data. MF = mixed forest, TWL = transitional woodland, CF = conifer forest, Fields = fields and agricultural land, BL = broad-leaved forest, Peats = peatland, WB = water bodies, HI = housing, OI = other infrastructure

several lynx did not use at all. Therefore, we omitted peatlands from the results (Fig. 5). A drop in the number of individuals that actually had used the habitat ($N = 16/22$) and the high use of peatland by a few individuals apparently inflated the result of this habitat compared to its availability, resulting in false overall selection.

Table 5 Habitat ranking matrix based on the parameter estimates of the resource selection functions with significance levels based on pairwise comparisons between the habitats. The upper triangle on the right is the daytime selection and the lower left triangle is the night-time selection (+/− = habitat on the row selected more/less than habitat in the column, +++/−−− = selected significantly more/less)

	TWL	MF	BL	CF	Fields	OI	Peats	HI	WB
TWL		−	+	+++	+++	+	+	+	+++
MF	−		+	+++	+++	+	+	+	+++
BL	−	−		+	+++	+	+	+	+++
CF	−−−	−−−	−		+++	+	+	+	+++
Fields	−−−	−	−	+		+	+	+	+++
OI	−	−	−	−	−		+	+	+
Peats	−	−	−	−	−	−		−	+
HI	−−−	−	−	−	−	−	−		+
WB	−−−	−−−	−−−	−−−	−−−	−	−	−	

Key: *TWL* transitional woodland, *MF* mixed forest, *BL* broad-leaved forest, *CF* conifer forest, *Fields* fields and agricultural lands, *OI* other infrastructure, *Peats* peatland, *HI* housing, *WB* water bodies

Discussion

We studied movement patterns and habitat use and selection during Eurasian lynx natal dispersal in Finland in a population without known dispersal barriers. Habitat use and selection by dispersing Eurasian lynx have not been previously studied. The typical timing for the onset of dispersal was April, which was close to the birth of a new litter in late May to early June. The onset ages did, however, vary between 7 and 16 months, agreeing with those known from other European populations that were typically 9 to 11 months (Breitenmoser et al. 1993; Schmidt 1998; Zimmermann et al. 2005; Samelius et al. 2012) (between 6 and 18 months).

In our study, there was large individual variation in natal dispersal distances and duration regardless of sex. However, we acknowledge that our sample size was most likely too small to detect any sex bias in dispersal. According to our previous genetic study, lynx females seem to form spatial matrilinear clusters, suggesting at least partial philopatry (Holmala et al. 2018). In Scandinavian telemetry studies, the maximum dispersal distances were shorter for females than

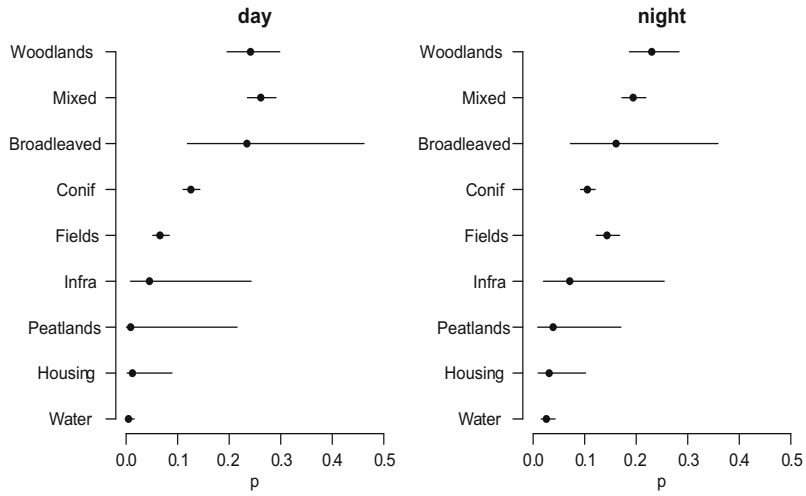

Fig. 4 Estimates and 95% confidence intervals of habitat selection indicators derived from the estimated resource selection functions. The displayed values p_{jk} for each habitat j (y-axis) and time-of-day k (daytime left nighttime right) can be interpreted as probability to select habitat j at time-of-day k if all habitats were equally available for dispersing Eurasian lynx (sexes pooled)

males (although the range was wide for both sexes), and a third of females remained philopatric (Samelius et al. 2012). Our results provided information about those females that do disperse, which remains a common trait even when a majority may remain philopatric (Holmala et al. 2018). Long-range dispersal has been associated with a species' range expansion (Thompson and Jenks 2010) and underlying, complex local population dynamics, while stable dynamics select for short-distance dispersal (Murrell et al. 2002). Local population stability possibly differs between sexes and may thus be difficult to differentiate from inbreeding avoidance or simply the social organization. Moreover, perturbations may be affected by intensive hunting which creates vacant home ranges.

To our knowledge, this is the first time the actual route travelled during dispersal was studied in this level of detail, as other papers on lynx dispersal have only reported the distances between the start and the finish, partly because the technology used previously (mainly VHF tracking) has not permitted the description of the routes. The dispersal distances of the Finnish lynx seem to fall somewhere between the dispersal distances of Scandinavian and Central European lynx. The maximum distances in Finland were substantially shorter than those in Scandinavia (Samelius et al. 2012), probably because of the vast mountainous areas with little canopy cover in northern Scandinavian landscapes. While the distance between the start and finish may provide information about

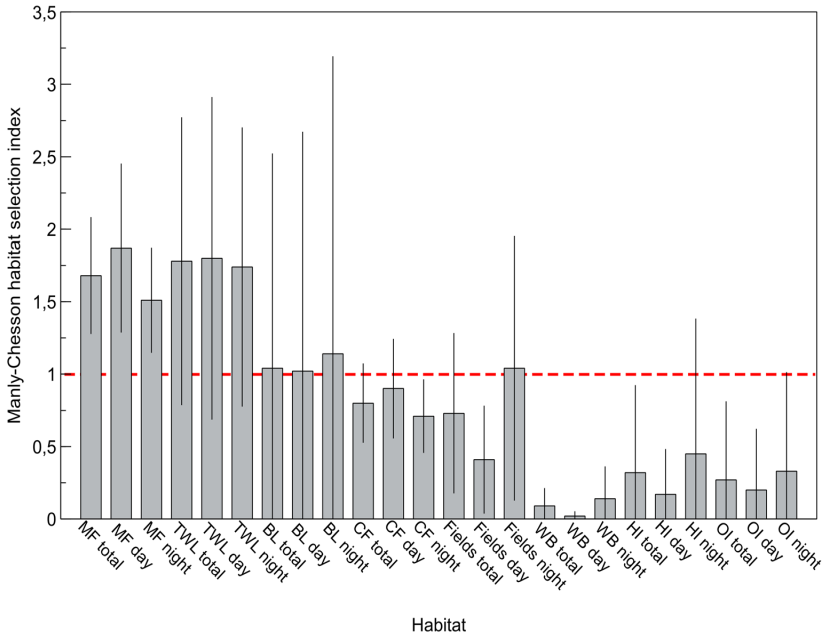

Fig. 5 The Manly-Chesson habitat selection index for the Eurasian lynx during dispersal. Values = 1 indicate the habitat usage in proportion to availability values > 1 indicate habitat preference and values < 1 indicate avoidance. The error bars represent the standard errors. Peatlands were omitted from the figure because of their extremely low availability. MF = mixed forest, TWL = transitional woodland, CF = conifer forest, BL = broad-leaved forest, Fields = fields and agricultural lands, WB = water bodies, HI = housing, OI = other infrastructure

where the lynx settles, it does not tell much about the dispersal per se, as according to our results, an average of 80% of the dispersal route resulted from movement other than the straight-line distance between the start and the end points. An extreme case was female F7, which dispersed 1178 km but ended up only 30 km from its onset point.

The dispersal speed was the highest in the evening and at night, although the lynx did travel during the day as well. In a low-density lynx population in Norway, female subadults moved 2.5 km per day (mean), which was similar to daily movement distances by resident females (2 km) but less than half of the daily distance of resident males (5.9 km) (Sunde et al. 2000). Male lynx home ranges in Norway are approximately three times larger than those of females, so the daily movement distances were in line with the home range sizes (Sunde et al. 2000), as was movement speed to route length shown in our study. The daylight period varies tremendously with season and spatially within Finland. An individual dispersing during spring and summer may only experience a few hours of darkness per day, whereas an individual dispersing in autumn or winter may only experience a few hours of daylight per day, making comparisons between the different light conditions statistically unsound. That is why we settled for using the division based on time-of-day (GMT).

The lynx in our study used mostly forest habitats but some of the habitats were used disproportionally to their availability. The lynx selected mixed forests during both night and day, whereas the conifer forests were used mainly during the night. The heterogeneity of the tree species and thick undercover in mixed forests likely attracted more prey than a commercial conifer forest and provided ample ambush and hiding places for lynx. In studies of habitat use and selection involving resident Eurasian lynx, a preference for forests has been documented (Niedziałkowska et al. 2006; Basille et al. 2013; Filla et al. 2017). In Poland, resident lynx selected for high complexity forest habitats with low visibility and used conifer forests less than expected (Podgórski et al. 2008).

The studied, dispersing lynx selected areas with sparse tree-cover in transitional woodlands. These habitats included sapling stands and early successional vegetation, which attract many small mammal species (Kirkland 1977; Swanson et al. 2011). Pine and spruce saplings also provide plenty of cover. Even though open areas were not selected, fields and agricultural lands were selected during the active hunting period at night. The Finnish agricultural landscape is a relatively small-scale mosaic that provides many potential hiding places for an ambush predator and could, like transitional woodlands, also be described as habitat with high ease of prey capture.

Dispersing lynx avoided human settlement and other human infrastructure, which included roads in our study. These results are in line with previous studies on resident lynx habitat selection (Bunnefeld et al. 2006; Basille et al. 2008, 2013; Filla et al. 2017). The lynx selected the habitat more carefully during the day than during the night, which is reflected as statistically significant interaction effect of habitat and time-of-day and as smaller differences between the estimated habitat selection indicators in the night-time than during the day (Table 5). Filla et al. (2017) found that resident lynx in Central Europe selected for more rugged, closed terrain away from human infrastructure during the day and open habitats during the night, presumably due to opportunities for hunting. The avoidance of open habitats was weaker during twilight and night in a study by Gehr et al. (2017). The lynx in their study responded to the trade-off between the prey availability and human presence by selecting high prey availability areas during times of low human activity (Gehr et al. 2017).

Our study shows that the lynx select for and between tree-covered habitats, also when the landscape is mostly composed of these habitats (totalling almost 70% of the study area). This could imply that for example in Central Europe, the scarceness of tree-covered habitats effectively emphasises their significance for the dispersing lynx, therefore playing a fundamental part in connecting populations and lynx conservation.

Future studies should refine the understanding of the arrangements of vacant and non-vacant home ranges in the landscape. It is crucial to incorporate ecological and genetic methods to gain better insight of the dispersal behaviour, especially the local population structure in relation to that. It is possible that young lynx caught for research do not represent the dispersers of the total population, as has been pointed out by others regarding for example the sex ratios, behavioural traits of caught individuals and biases caused by trapping methods (Tuyttens et al. 1999; Lofroth et al. 2008; Conde et al. 2010; Stuber et al. 2013). These discoveries support the need of using different methods for catching lynx or finding alternative tracking methods altogether.

In conclusion, Eurasian lynx dispersal occurred in the crepuscular and night-time activity pattern that is typical of the species. While dispersing, young lynx seemed to select their movement habitats so that they offered cover, security and hunting possibilities. Our study could not conclude anything on the sex bias in dispersal because of high individual variation and relatively few data on separate sexes.

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Data availability The data that support the findings of this study are available from Luonnonvarakeskus (Natural Resources Institute Finland, Luke), but restrictions apply on the availability of these data, which were used under the licence for the current study, and so are not publicly available. Data are, however, available from the authors upon reasonable request and with the permission of Luke.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The handling protocol of lynx was approved by the Finnish Animal Ethics Committee (ELLA) and fulfils the ethical requirements for research on wild animals in Finland. In addition, permits for wild animal capture were obtained from the Finnish Wildlife Agency and Finnish Ministry of Agriculture and Forestry.

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II

Genetic analysis indicates spatial-dependent patterns of sex-biased dispersal in Eurasian lynx in Finland

Manuscript

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Short title: Sex-biased dispersal in Eurasian lynx

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Abstract

Conservation and management strategies for large carnivores require knowledge of female and male dispersal mechanisms and rates. This knowledge is challenging to obtain and often incomplete or contradictory, where results may be influenced both by the methods used and the size (and connectivity) of the population, the study area, and the sample material. We have genotyped Eurasian lynx (180 males and 102 females, collected 2003-2017)

continuously distributed in southern Finland (~23.000 km^2) using 21 short tandem repeats (STR) loci and compared statistical genetic tests to infer local and sex-specific dispersal patterns within and across both genetic clusters and geographic regions. We tested for sex-specific substructure with individual-based Bayesian assignment tests and spatial autocorrelation analyses. Differences between the sexes in genetic differentiation, relatedness, inbreeding, and diversity were analysed using population-based AMOVA, F-statistics, and assignment indices. Our results showed two different genetic clusters that were spatially structured for females but admixed for males. Similarly, spatial autocorrelation and relatedness was significantly stronger in females than males. However, we found weaker sex-specific patterns for the Eurasian lynx when the data were separated in three geographical regions than when divided in the two genetic clusters. Overall, our results suggested male-biased dispersal and female philopatry for the Eurasian lynx in Southern Finland, with increasing strength from west to east within our study area. In addition, we conclude that our detection of male-biased dispersal was dependent both on analytical methods utilized, on whether subtle underlying genetic structuring was considered or not, and the choice of population delineation. Conclusively, we suggest using multiple genetic approaches to study sex-biased dispersal to obtain robust results when analysing continuous populations, in which population delineation is difficult.

Key words: *Lynx lynx*, genetic relatedness, sex-biased dispersal, philopatry, continuous populations

Introduction

Individual dispersal into a breeding area impacts the evolutionary potential, long-term viability, and distribution ranges of populations and species (Johnson and Gaines 1990, Bowler and Benton 2005, Driscoll et al. 2014, Cayuela et al. 2018, Hanski 1991) [1-5]. This type of dispersal is often sex-biased to avoid potential breeding of closely related individuals (e.g., Saastamoinen et al. 2018, Li and Kokko 2019b) [7,8]. Avoidance of inbreeding increases an individual's fitness, but also affects the social and spatial genetic composition of a population (Greenwood 1980, Bowler and Benton 2005, Cayuela et al. 2018, Saastamoinen et al. 2018, Li and Kokko 2019b) [2,4,6-8]. Successful immigrants may introduce new alleles and thereby increase genetic material and effective population size by enlarging the area from which partners are drawn (Frankham 1995, Cayuela et al. 2018, Saastamoinen et al. 2018) [4,5,9]. Dispersal varies with several factors, including population size, landscape features, various anthropogenic disturbances, and especially by social systems of the species (Rueness et al. 2003, Holmala et al. 2018) [10,11]. A detailed understanding of species-specific dispersal is required to forecast population responses to environmental changes (Bowler and Benton 2005, Driscoll et al. 2014) [2,3].

Large terrestrial carnivores are often reported to display sex-biased dispersal (e.g., Lawson Handley and Perrin 2007, Costello et al. 2008, Li et al. 2019b, Croteau et al. 2010, [8, 18, 23-25]. In the *Lynx* family, sex-biased dispersal has been reported for bobcats (*Lynx rufus*; Kitchings and Story 1984, Janečka et al 2007, Croteau et al 2010) [24,29,30], but not for Canada lynx (*Lynx canadensis*) or Iberian lynx (*Lynx pardinus*; Poole 1997, Campbell and Strobeck 2006, Ferreras et al 2004) [31-33]. Studies of Eurasian lynx with telemetry have documented dispersal of both sexes but cannot consistently ascertain that dispersal is sex-biased. In a Scandinavian telemetry study, males dispersed further and more frequently than females, of which one third remained philopatric (Samelius et al 2011) [34]. In a Polish

telemetry study, the few males studied predominantly dispersed further than the females (Schmidt 1998) [35]. In two Swiss studies, both sexes showed similar dispersal parameters using telemetry, but rather differed between areas of origin with sex as an additional factor (Zimmermann et al 2005, 2007) [36,37]. Genetic studies can detect the amount and direction of sex-biased gene flow
as an indicator of sex-biased dispersal (Lawson, Handley and Perrin 2007) [8]. In a genetic study in Poland, local females were more closely related to each other than males (Schmidt et al 2016) [38]. Furthermore, in a small, isolated population in the western Carpathians, the genetic analysis showed that females were predominantly philopatric and reproduction was dominated by a few males, leading to inbreeding and sub-structuring into two family lineages (Krojerová-Prokešová et al. 2018) [39]. Previously, we have detected local family genetic structuring in female Eurasian lynx continuously distributed in Finland, but it remained unclear whether this suggested philopatry as only females were investigated (Holmala et al. 2018) [11]. In a large-scale study including several lynx populations, similar decreasing relatedness with increasing distance on a population level was observed for both sexes (Ratkiewicz et al. 2014) [40].

There may be several reasons for the observed variation across studies considering Eurasian lynx dispersal. The Eurasian lynx populations in Europe differ in size and continuity (von Arx, Breitenmoser-Wuersten, Zimmermann, & Breitenmoser, 2004) [???], but many populations are small and fragmented (Kaczensky et al., 2013) [???]. Additionally, the different methodologies applied may influence the detection of sex-biased dispersal (e.g., telemetry *versus* genetic, see Driscoll et al. 2014, Prugnolle and de Meeus 2002, Goudet et al. 2002) [3,26,27], as do the statistical approach taken (Favre et al. 1997, Goudet 2002, Banks and Peakall 2012) [???], and the temporal and geographical scales studied (Morton et al.

2018) [28]. Sex-biased dispersal may be studied using statistical genetic methods based on different principles. Broadly, these fall into two categories: i) population-based methods, which rely on pre-defined populations, using F-statistics (Weir & Cockerham 1984; Favre et al. 1997) [42-43], analyses of molecular variance (Excoffier et al. 1992) [44], and assignment indices (Favre et al. 1997, Goudet 2002) [43,45]; and ii) individual-based methods, which do not require pre-defined populations, such as spatial autocorrelation analysis (Banks and Peakall 2012) [41] and Bayesian assignment methods like STRUCTURE (Pritchard et al. 2000) [46]. The latter also allows immigrant and admixed individuals to be comparatively easily identified (Lawson Handley and Perrin 2007, Manel et al. 2005) [18,47].

In this study, we have used genetic approaches of both category i) and ii) to infer sex-specific dispersal patterns from genetic data in a continuously distributed population of Eurasian lynx in southern Finland. We applied 21 bi-parentally inherited short tandem repeat (STR) markers to individuals sampled after dispersal (> 1 year), to obtain unbiased estimates (Lawson Handley and Perrin 2007) [18] and high power of tests for sex-biased dispersal (e.g., Prugnolle and de Meeus 2002) [26]. By building upon the results of our previous study on female Eurasian lynx (Holmala et al. 2018) [11], that documented matrilineal assemblages in the study area, we tested the null hypothesis of equal dispersal of sexes by estimating the degree of sex-specific patterns in dispersal, spatial organization, and genetic diversity indices. Our prediction was that males will contrast the patterns in females by displaying a significantly higher degree of dispersal, and thus show lower genetic differentiation, clustering, assignment indices, and relatedness than females. However, we also investigated how population- and individual-based genetic tests of sex-biased dispersal differ in their power and bias depending on the groups selected for analysis, i.e. genetic clusters or arbitrarily pre-defined geographical regions (Lawson Handley and Perrin 2007, Manel et al. 2005) [18,47].

Materials and Methods

Study area, sampling and age estimation

Our study area is in southern Finland (Fig 1), covering a total area of 22,936 km^2. Forest dominated by pine, spruce, and birch constitutes about 70% of the terrestrial area. Lakes encompass about 10% and the rest consists of mires, farmland, and urban areas, creating a blend of different land uses. Despite the high forest cover, the area holds five of the 15 biggest towns in Finland, including the capital area of Helsinki, which lies about 100 km east from the western-most point of the study area. Human population density varies between 6 to 3044 individuals / km^2. Lynx recolonization from east to west took primarily place before the sampling period of this study (2003-2017, Kojola et al. 2005 [48]. The lynx population is continuous and connected with that of Russia (Ratkiewicz et al. 2014) [40]. Yearly derogation hunting of Finnish lynx is regulated by the Ministry of Forestry and Agriculture and has varied from about 10 - 20 % of the estimated population size during our sampling period (Luke 2018) [49]. Our initial data set consisted of all 369 legally hunted lynx during 2003-2017 (238 males and 131 females). Tissue samples for genetic analysis were collected from all individuals at the Natural Research Institute Finland's (Luke) lab in Taivalkoski Research Station. No ethic permit was required, as the samples did not involve live animals. A CITES permit was obtained for shipping the samples to the Norwegian Institute of Bioeconomy Research (NIBIO) lab in Svanhovd, Norway. The tissue samples were stored in ethanol and kept frozen until analysis. The age of the individuals was determined from cementum annuli analysis of tooth samples by Matson´s Laboratory, Montana (Matson, 1981) [50]. Before the analyses of sex-biased dispersal, 0-year olds were excluded to avoid the analysis for sex-bias to be affected by undispersed individuals (Prugnolle & de Meeûs 2002) [26]. This resulted in a dataset (n = 282) consisting of 180 males and 102 females (Table 1; Fig. 1).

Fig 1. The study area for Eurasian lynx in Southern Finland. Spatial distribution of the two genetic clusters for both male and female Eurasian lynx (N_{male}= 180; N_{female}= 102) as well as admixed individuals in black. Black boxes indicate the three geographical regions used for the LOCPRIOR function in STRUCTURE and as an alternative grouping for summary statistics studied.

DNA extraction and genotyping

Procedures for DNA extraction and genotyping are outlined in detail in Holmala et al. (2018). Briefly, after DNA extraction from tissue samples with the DNeasy Tissue kit (Qiagen), samples were genotyped at 21 short-tandem repeat loci (STRs; Menotti-Raymond et al. 1999, Carmichael et al. 2000) [51,52] in seven multiplexes (Holmala et al. 2018) [11]. Compared to Holmala et al. 2018 [11], the use of two STR markers (Fca078 and Fca001) was discontinued in the present study due to impractical allele scoring. Molecular sex determination was done by amplifying regions on the zinc finger region on the felid X- and Y-chromosome with primers developed by Pilgrim et al. (2005) [53]. Amplification reactions contained 5.0 µl 2x Multiplex PCR MasterMix (Qiagen), 1.0 µl Primer mix, 0.05 µl BSA (NEB) and 2.95 µl ddH2O in a 10 µl PCR reaction with 1µl template DNA. The PCR thermocycling protocol included the following steps: 10 min at 95°C followed by 29 cycles of 30 s at 94°C, 30 s at 56/57/58/59°C (annealing temperature differed among multiplex sets, see Holmala et al. 2018) [11], 1 min at 72°C, with a final elongation step of 45 min at 72°C. An ABI PRISM 3730 sequencer was used to analyse samples and GeneMapper v4.1 (Applied Biosystems) for subsequent genotyping. For assessment of genotyping reliability, 10% of the samples were chosen randomly and analysed a second time.

Genetic summary statistics

We used Micro-Checker 2.2.3 (van Oosterhout et al. 2006) [54] to detect the potential presence of null alleles and scoring errors due to large dropout alleles or stutter. Tests for

linkage disequilibrium and deviations from Hardy-Weinberg expectations were carried out both at a genetic cluster and geographic region level in Genepop 4.7.0 (Rousset 2008) [55] with the following parameter settings: dememorization = 10,000, batches = 5,000, and iterations per batch = 10,000. Finally, GenAlEx 6.51b2 (Peakall and Smouse 2006, 2012) [56,57] was used to estimate genetic summary statistics (i.e., observed and expected heterozygosity, and inbreeding coefficient) for all groups analysed.

Population genetic structure

We applied a Bayesian clustering algorithm using the software package STRUCTURE 2.3.4 (Pritchard et al. 2000, Falush et al. 2003) [46,58] to assess overall and sex-specific genetic clustering. The entire dataset of 369 individuals was initially used, without any pre-defined populations, to obtain the most reliable assignment scores possible, which were subsequently utilized for sorting individuals into genetic clusters and admixed groups after removal of 0-year olds to limit the analysis of sex-biased dispersal to adult specimens in subsequent analyses. In addition, the reduced data set was run separately to ensure that the number of K was consistent between full and reduced data set. Using the correlated allele frequency model and assuming admixture, forty independent runs of K = 1 - 5 were carried out with a burn-in of 100,000 and 1,000,000 MCMC repetitions. The runs were conducted on the CIPRES Portal v3.3 at the San Diego Supercomputer Center (https://www.phylo.org/; Miller et al. 2010) [59] that permits for parallelised computation with the R package PARALLELSTRUCTURE (Besnier et al. 2013) [60] to reduce computation time. We processed STRUCTURE results with CLUMPAK (Kopelman et al. 2015) [61]. Following Evanno et al. (2005) [62], the modal value of the ad hoc quantity ΔK was used as the criterion to infer the most likely number of genetic clusters. A second set of analyses served as an additional test to determine the most likely number of K and was based on arbitrarily pre-defined populations along the distribution continuum. We did this by pooling the

individuals according to their sampling location into three regions; western, central, and eastern, each containing 1/3 of the samples (Fig 1). Since we had pre-defined populations in this case, we ran these analyses twice, once without and once using the LOCPRIOR function; the latter incorporates spatial information as a prior in the model. With the LOCPRIOR, the clustering algorithm assumes that a probability of an individual to be assigned to a cluster varies according to its sampling location, decreasing the potential of finding false population structuring (Hubisz et al. 2009) [63].

Spatial autocorrelation

We performed spatial autocorrelation analyses using GenAlEx 6.51b2 with 9,999 permutations (Peakall and Smouse 2006, 2012) [56,57] to compare the pairwise relationship between genetic and spatial distance between males and females. We used 15 km distance class for both sexes, because tests of other distances classes (i.e., 5, 10, and 20 km; data not shown) gave nearly identical results for males and because 15 km was already determined to be appropriate for females in Holmala et al. (2018) [11]. Because pooling data from genetically differentiated clusters may potentially bias the overall genetic correlation coefficient, *r*, we ran the analysis on the regional level (all genotypes within each sex) using the multiple genetic cluster approach (Holmala et al. 2018) [11], treating the detected genetic clusters and admixed individuals as groups. Additionally, we also ran the analysis separately for male and female genotypes both within clusters assigned by STRUCTURE and by dividing the data set into three geographical regions to conclude whether the possible bias is spatially variable.

Analyses of Molecular Variance

We performed an AMOVA (Analyses of Molecular Variance, Excoffier et al. 1992) [43] to investigate differences between sexes in levels of genetic variation partitioning among and

within both STRUCTURE clusters and the three geographical groups as described above to test whether the exclusion of admixed individuals and first-generation migrants or spatial overlap of clusters affected our results.

Assignment indices and genetic diversity estimators

Assignment indices calculate the probability of an individual's multilocus genotype originating in the population in which it was collected (Favre et al. 1997, Prugnolle and de Meeus 2002, Goudet et al. 2002) [26,27,44]. The dispersing sex should therefore display negative assignment indices while the philopatric sex displays positive indices. Further, under sex-biased dispersal, individuals of the dispersing sex are expected to show lower R-values (Prugnolle and de Meeus 2002) [44], F_{ST}-values, and observed heterozygosity-values (i.e., Wahlund effect) and consequently, higher F_{IS}-values than the philopatric sex (Goudet et al. 2002) [27]. We assessed differences between sexes in five genetic diversity estimators (i.e., Relatedness (r), genetic differentiation (F_{ST}), inbreeding coefficient (F_{IS}), mean (mAIc) and variance (vAIc) of the corrected assignment index AI (AIc) with the software package FSTAT 2.9.3.2 (Goudet 1995, Favre et al. 1997, Goudet 2002) [27,44,45] and tested for significance using a permutation test with 5,000 iterations. In addition, we also estimated AIc and mAIc based on the methodology by Favre et al. (1997) [44] and extended by Mossman and Waser (1999) [64]. Significance was determined with a nonparametric Mann Whitney U-test (MW-U) in GenAlEx 6.51b2 (Peakall & Smouse 2006, 2012) [56,57]. All tests were run twice; first on the genetic clusters as retrieved by the STRUCTURE analysis and second, on individuals pooled according to the predefined geographical regions.

Results

Genotyping and genetic variation

Genetic variation was found to be relatively high for the population (n=282) with average expected and observed heterozygosity of 0.651 and 0.662 (Table 1). The observed and expected heterozygosity and inbreeding coefficients, showed no signs of inbreeding, except for Fca-077, or large differences between observed and expected heterozygosity either at the locus level or at the genetic cluster and geographical group level (Table 1, 2). There was no indication for scoring errors due to stutter or large allele dropout. Only one locus showed significant evidence for null alleles (Fca-077 in GR3) and therefore, overall evidence for null alleles was considered low. All loci were polymorphic and in Hardy-Weinberg equilibrium after Bonferroni correction, except Fca-077 in (eastern) geographical region 3 and genetic clusters 1 and 2. There was no evidence for linkage disequilibrium after correction for multiple testing and 90/630 pairwise comparisons were significant at $p = 0.05$ level based on the three geographical regions. At the genetic cluster level, 84/630 tests were significant at the 0.05 level, of which three remained significant after Bonferroni correction.

Population genetic structure

For all three data sets (i.e., sexes combined, females, and males), two distinct genetic clusters were identified by the Evanno method when running the analysis without predefined populations (Evanno et al. 2005; Fig 2A-C) [62]. For sexes combined and females, the clusters displayed a gradual shift from west to east in relative distribution across the study area (Fig 2G and H). In contrast, males showed complete spatial admixture (Fig 2I). Although a model of three genetic clusters received lower support, the STRUCTURE bar plots indicated the existence of a third genetic cluster in females, located in the eastern part of the study area. The STRUCTURE analysis using the LOCPRIOR function based on

geographical regions was consistent with this interpretation, supporting differential genetic structuring between sexes (Fig 2J).

Fig 2. Spatial genetic structure identified by Bayesian cluster assignment analysis with STRUCTURE for Eurasian lynx in southern Finland. Fig 2A-C show the DeltaK plots for 2A) the combined data set, 2B) females, and 2C) males, respectively. Fig 2D-F display the mean of estimated log-likelihood values for 2D) the combined data set, 2E) females, and 2F) males. Fig 2G-J. CLUMPAK-averaged Bayesian clustering (STRUCTURE) plots showing posterior probabilities of Lynx lynx individual genotypes (as bars) assigned to each genetic cluster based on STR data for K = 2 - 3. Individuals are sorted by geography from west to east in STRUCTURE bar plots. Fig 2G) sexes combined (N = 282), 2H) females (N = 102), and 2I) males (N = 180). In addition, Fig 2J) shows the bar plot for females across three geographical regions as retrieved from a STRUCTURE run with the LOCPRIOR option.

Spatial autocorrelation

Across genetic clusters, females showed significant decreasing spatial autocorrelation up to 45 km ($r_{females}$ = 0.106; Fig 3A), whereas males showed no such pattern (r_{males} = -0.002; Fig 3B). This difference between the sexes was apparent also within genetic clusters (Fig 3C, D), although non-significant for females in cluster 1 ($r_{cluster1}$ = 0.055, $r_{cluster2}$ = 0.121, $r_{admixed}$ = 0.142; Fig 3C). Males showed no spatial autocorrelation in any of the genetic clusters ($r_{cluster1}$ = -0.022, $r_{cluster2}$ = -0.006, $r_{admixed}$ = 0.006; Fig 3D). Across geographic regions, a similar pattern emerged; however, the estimated sex-bias was reduced with more than 50% compared to the cluster-based analysis ($r_{females}$ = 0034, r_{males} = -0.004, Fig 3E, F). Similarly, within geographical regions, females displayed consistently lower spatial autocorrelation estimates (r_{GR1} = 0.034, r_{GR2} = 0.030, r_{GR3} = 0.062; Fig 3G) than within genetic clusters, while males again showed no spatial autocorrelation at all (r_{GR1} = -0.003, r_{GR2} = -0.004, r_{GR3} = -0.004; Fig 3H).

Fig 3. Spatial autocorrelation within geographic distance classes for genetic clusters and geographical groups for the Eurasian lynx in southern Finland 2003-2017. Fig 3A) Combined spatial structure analysis for females in both genetic clusters and the admixed individuals. 3B) Combined spatial structure analysis for males in both genetic clusters and the admixed individuals. 3C) Spatial autocorrelation for females in both genetic clusters and admixed individuals. 3D) Spatial autocorrelation for females in both genetic clusters and admixed individuals. genetic cluster 1 = blue line, genetic cluster 2 = orange line, admixed individuals = grey line. F = females, M = males. 3E) Combined spatial structure analysis for females based on geographical regions. 3F) Combined spatial structure analysis for males based on geographical regions. 3G) Spatial autocorrelation for females in the three geographical regions. 3H) Spatial autocorrelation for males in the three geographical regions. Geographical region 1 = blue line, geographical region 2 = orange line, and geographical region 3 = grey line. The 95% confidence interval for the Null hypothesis of random distribution is given as a dashed line, the bootstrap errors are displayed as whiskers.

Analyses of Molecular Variance

When comparing the molecular variance among both identified genetic clusters and the group of admixed individuals in an AMOVA analysis (Table 3), significant and similar between-cluster differentiation was found both overall and in females and males separately. In contrast, the analysis based on geographical regions gave similar results as the sex-specific STRUCTURE analysis (Table 3) in that lower partitioning of molecular variance across spatial groups was found in males than in females, consistent with male-biased dispersal.

Assignment indices

The results from FSTAT analyses showed significantly lower mAIc (mean Assignment Indices corrected) values with higher variance (i.e., vAIc, Table 4) in males than females.

However, the difference between sexes was larger in genetic cluster-based analysis than in geographical region-based analyses (Table 4). Consistent with this, taking out female individuals that grouped in a third separate cluster only found in the eastern part of the study area in the STRUCTURE analysis (Fig 2H) also made the sex-bias clearer (results not shown). Similarly, GENALEX analyses showed a higher frequency of negative AIc-values (Fig 4A) and lower mean AIc-values (Fig 4B) in males than females, although the latter difference was not statistically significant unless removing the females that grouped in a third separate cluster (Fig 2H). Although non-significant, the strength of sex-bias increased when removing females in cluster 2 (results not shown).

Fig 4. Frequency distribution and mean of corrected genetic assignment indices (AIc) for lynx males and females of Eurasian lynx in southern Finland 2003-2017. Fig 4A) Frequency distribution of AIc (corrected Assignment Index) values in the entire data set. Data for males shown in black bars and data for females shown in white bars. Fig 4B) Mean AIc values in males and females.

FSTAT genetic diversity estimators and genetic relatedness

All FSTAT estimators showed outcomes expected for the philopatric and dispersing sex, respectively, albeit only three of the seven estimators were statistically significant at the cluster-based level (i.e., mAIc, vAIc, and H_S) and two additional estimators at the geographical region level (i.e., F_{ST} and REL, Table 4).

Thus, the majority of FSTAT estimators supported male-biased dispersal, particularly the analysis based on geographical regions. Group-based estimates of relatedness were on average higher when based on genetic clusters than on geographic regions. While the genetic clusters showed no consistent difference in relatedness between sexes, two out of three geographical regions showed significantly higher relatedness between females than males

(Fig 5B). The central region (GR2) and the admixed group showed the lowest average relatedness estimates and no difference in relatedness between sexes.

Discussion

We found clear genetic evidence of male-biased dispersal and female philopatry in Eurasian lynx continuously distributed in Southern Finland, with increasing strength from west to east within our study area. This spatial gradient in sex-biased dispersal indicated regional differences in the spatial organization of female relatives, since no spatial patterns was observed for males. Furthermore, the detection of sex-biased dispersal in this continuous population was dependent on analytical methods utilized, which power and estimate sizes were often (differentially) sensitive to whether subtle underlying genetic structuring was considered or not. Males showed spatial admixture between genetic clusters, while females were spatially structured. Spatial autocorrelation and relatedness were also significantly stronger in females than males. However, we found weaker sex-specific patterns when the data were separated in three geographical regions than when divided in the two genetic clusters. Assignment indices were also consistently larger when the analysis was based on genetic clusters than on geographic regions. In contrast, molecular variance showed higher structuring for females for the three geographical regions than for the two genetic clusters. FSTAT estimators supported male-biased dispersal, particularly the analysis based on geographical regions. Thus, the detection of sex-biased dispersal was dependent both on analytical methods utilized, on whether subtle underlying genetic structuring was considered or not, and the choice of population delineation.

Methodological considerations

Both individual-based methods in category ii) clearly indicated male dispersal and female philopatry. Although seldom applied, Bayesian individual assignment methods like STRUCTURE may thus reveal sex-specific structure and help define genetic clusters to be

used in population-based methods (see also e.g., Schregel et al. 2017) [65]. Similarly, the power of spatial autocorrelation analysis peaks at the scale where clustering of related individuals is the highest and correcting for overlapping genetic clusters, this may have contributed to the detection of sex-biased dispersal in this study. Hence, in situations like this, a correction for spatial genetic structure may be advisable to detect subtle sex-biased dispersal patterns. For large carnivores and other species that have large home ranges and continuous populations as demonstrated here, our results suggest that that the combination of the two individual-based genetic approaches may provide complementary information.

In addition, three different population-based approaches were used (category i)). While the AMOVA based on geographical regions indicated higher structuring and philopatry in females than in males, the cluster-based analysis showed similar estimates for both sexes. This suggests that the latter approach may underestimate the sex-bias if the genetic clusters show spatial overlap, since genetic variation between sexes may be geographically distributed more evenly than among genetic clusters. Excluding admixed individuals and first-generation migrants (which are presumably more common in the dispersing sex) in the genetic cluster analysis may also lead to a downwards bias in the molecular variance difference between the sexes. Therefore, AMOVA is probably most effective if applied directly on geographical sampling locations in continuous overlapping populations. For the assignment indices, we found that that even subtle sex-specific substructure can reduce the power of the analysis for sex-biased dispersal testing. Female individuals from a different genetic cluster that were located only in part of the study area, lowered the positive assignment index, both overall and particularly in cluster 2/geographical region 3 as those females were identified as immigrants. This bias seems particularly affecting this test while, for example, relatedness estimates per group were only slightly changed (results not shown) when taking cluster 3 females out of the

analyses, indicating that summary statistics may be less prone to the presence of immigrants than assignment indices.

Some tests of sex-biased dispersal are relatively less powerful, such as F_{IS} (Goudet et al. 2002) [27], which was confirmed in our study regardless of the groupings assessed. Being closely linked to F_{IS}, the same applies to the values of observed heterozygosity according to our results. Based on tests by (Goudet et al. 2002) [27], F_{ST} and relatedness perform particularly well when the proportion of dispersers is high in the sample, even with low genetic differentiation among groups. This may be better captured in the geographical region approach in the current study, by likely including a higher proportion of dispersers. In contrast, estimators like mAIc, vAIc, and H_S appear less sensitive to this type of bias and may more readily be applied to genetic clusters identified by Bayesian individual assignment tests. Finally, sampling bias can also influence FSTAT estimators, which significantly reduces their power if it reaches a ratio of 80:20 (Goudet et al. 2002) [27]. In general, hunting bags included more males than females (Luke 2018) [49], and the ratio in our study was 64:36 M:F, if all males in our study dispersed and all females assigned to a cluster were philopatric, indicating that this did not downward bias the estimates of differences between sexes. To further test this, we subsampled the males and ran two additional analyses with GENALEX with two different male subsets, which resulted in higher female means as expected (results not shown). Therefore, we consider a potential sampling effect caused by the higher number of males as negligible.

Biological considerations

While earlier studies of Eurasian lynx reported conflicting evidence of sex-biased dispersal, we consistently found male-biased dispersal, which is typical for mammals (Li and Kokko 2019, Saastamoinen et al. 2018) [???]. The lynx population in southern Finland has increased

from 1,100 to 2,700 adult individuals during years 2007 to 2015 (Luke, 2017) [???] and represents a large and continuous population with active gene transfer with Russia (Chapron et al. 2014; Ratkiewicz et al. 2014) [???]. Habitat fragmentation can potentially reduce or eliminate dispersal-biases between sexes (Zimmermann et al. 2005) [36], by limiting population connectivity and reducing dispersal distances in high-density populations. Similarly, Tucker et al. (2017) [???] investigated the effects of spatial heterogeneity on genetic connectivity of species with sex-biased dispersal and found a stronger relationship between landscape features and genetic distance in the philopatric females in fishers. Thus, different land uses, and natural landscape heterogeneity may represent dispersal barriers for the philopatric sex. Although our study area was characterized by different land uses and levels of urbanization, our results were not indicative of any barriers to dispersal and our data on collared, dispersing lynx further supports this (Herrero et al. *in press*) [71] together with evidence regarding population health (Kareinen et al. 2020) [73] and active gene transfer (Ratkiewicz et al. 2014) [40]. Hence, anthropogenic and environmental changes that may trigger changes in sex-biased dispersal in this large Eurasian lynx population may be low compared to other studies.

Lack of dispersal barriers may also result in long-distance dispersal of both sexes (Dharmarajan et al 2009) [74]. Short-distance dispersers may be over-represented in the data if the study area does not reach beyond the species' dispersal capabilities (Koenig et al. 1996, Morton et al 2018) [28,77]. The degree of spatial genetic structure between individuals of the same sex may be affected by dispersal distance (Banks and Peakall 2012) [41] but could be diluted by behaviour (Zeyl et al. 2009) [78], or by emigration from genetically distinct populations (Biek et al. 2006) [79]. Our study area, while representing only a part of Finland, is 300 km in west-east and over 100 km in north-south-direction, which is well within the

dispersal distances for both sexes (Herrero et al. *in press*) [71]. Hence, we do not consider dispersal capabilities to be a major driving force in this study.

While short-distance dispersal works for the ultimate cause of inbreeding avoidance and kin competition, colonization of new patches is likely the cause for long-distance dispersal and hence independent of sex (Perrin & Goudet 2001, Ji et al 2009, Ratkiewicz et al. 2014) [40,74,75]. Using spatial autocorrelation, no evidence of scale-dependency of sex-biased dispersal (Vangestel et al. 2013) [67] was observed in our study. However, there was evidence of spatial variation: relatedness values (r) were relatively similar between sexes in genetic cluster 1 and geographic region 1, in the western part of the study area, but relatively different between in genetic cluster 2 and geographic region 3 (both in the east). Since males showed no spatial patterns on the scales studied, the difference observed between the sexes seemed to be due to variation in the spatial organization of related females, suggesting an overall stable pattern with potential regional differences influencing female philopatry.

The recolonization of lynx in Finland took place from east to west mainly before our sampling period, with the highest number of lynxes in 2014 (Luke 2015) [79]. In the east, the lynx population was established earlier, was stable, and mostly saturated (Holmala *unpublished*). In contrast, the western part of our study area was, at the time of sampling, still in the process of lynx recolonization, and thus probably did not represented a saturated population with a stable social organization (Holmala *unpublished*). These different colonization stages may explain the observed spatial variation in sex-specific autocorrelation, which was mainly due to spatial variation in the organization of the females caused by differences in population density and resource availability (Messier et al 2012, Li and Kokko 2019) [68,69]. Eurasian lynx shows large variation in dispersal distances, with both short and long-distance dispersal for both sexes (Breitenmoser et al. 1993, Schmidt 1998, Zimmermann et al 2005, Samelius et al 2011, Herrero et al. *in press*) [34,36,38,70,71]. In a dispersal model

by Perrin and Mazalov (2000) [72], competition for local mates strongly pressures males to disperse in polygynous species, whereas females only disperse if local resources, such as food and breeding sites are limited. Therefore, females greatly benefit from local knowledge in the natal area, and are discouraged from dispersing (Greenwood 1980, Li and Kokko 2019) [6,69], although female dispersal seemed to be slightly higher in the western than eastern part of our study area. Relatively high, male-biased hunting pressure on lynx in Finland constantly provides vacant territories weakening the competition between males, probably leading to less pressure to disperse (Ref?). We found no such spatial variation in male dispersal at the scales studied here. For females, hunting creates vacant territories for related females to occupy without the need to disperse further. Therefore, had we been able to sample the females that were not hunted as well, the observed pattern of philopatry could possibly be even stronger.

Ongoing anthropogenic pressures can considerably affect future connectivity and viability of the Eurasian lynx population studied here, and recolonizing large carnivore populations that display sex-biased dispersal more generally. Intensifying urbanization, agriculture, and forestry activities are all factors leading to increased habitat patchiness that is more likely to affect the philopatric sex (Tucker et al. 2017). Tucker et al. (2017) further demonstrated that different habitat features are important for female and male genetic connectivity. Therefore, management strategies need to consider these differences between the sexes and conserve landscape features facilitating dispersal of the philopatric sex. Indeed, our study shows that on these regional scales, female Eurasian lynx are more prone to show spatial variation in dispersal than are males.

If hunting targets the dispersing sex, like in Eurasian lynx in southern Finland, then reduced connectivity and increased population structure and inbreeding may result in the long run (Fattebert et al. 2015) [82]. Male Eurasian lynx have a higher chance of being hunted than

females, not only because of their trophy value, but also because of their behaviour (Bunnefeld et al. 2006) [83]. In our study, males showed no spatial variation in dispersal inferred from genetic data, suggesting that this is currently not an important factor in this large and relatively unfragmented population. Anthropogenic activities affecting the philopatric sex seems to be bigger potential threat, as our study indicated spatial variation in female organization due to unidentified biological or environmental influences. This may, in turn, lead to reduced population growth because individuals are either forced to choose badly or because of a discrepancy between true habitat quality and the available cues to perceive it (Kokko and Sutherland 2001) [84]. This may have negative effects for recolonizing large carnivore populations.

Conclusions

Research on the Eurasian lynx in the past have shown partly contradictory evidence concerning sex-biased dispersal. Two reasons for this discrepancy in findings include methodological and biological factors. We tackled the first issue by systematically applying comparative statistical analyses with different groupings that partially influenced the power of sex-biased dispersal detection. For species with large home ranges, generally continuous distribution ranges, for which populations cannot readily be delineated, and species that have high dispersal capabilities, we recommend combining a series of analytical approaches and groupings to evaluate potential biases of estimators. Further, careful consideration of how sampling biases may affect the results, is recommended.

We tackled the second issue by using continuous sampling and high-resolution genetic data on an appropriate geographic scale to capture differences in the strength of sex-biased dispersal across the study area. Our results point to highly flexible dispersal behaviour and spatial organization in female Eurasian lynx, which possibly reflects the local and regional

conditions of the population in question. Potential factors influencing the strength of sex-biased dispersal range from intrinsic factors such as density and adult turnover rate (Zimmermann et al. 2005, 2007) [36,37] to external factors like resource availability. Given the recent re-colonization history, our results are consistent with a less stable situation in the western part of the study area that may have weakened the signal of dispersal bias between males and females by affecting particularly the female part of the population. In addition, a mixture of anthropogenic activities, such as habitat alteration or hunting, may affect the structuring of individuals and possibly weaken the strength of sex-biased dispersal than would happen by social structure alone. However, there is currently little evidence that habitat fragmentation has had a major impact of dispersal in the current study. Yet, we cannot exclude the possibility that hunting pressures influenced the results in the current study in weakening the signal of sex-biased dispersal.

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Author Contributions

AH, CK, KH, and SH designed and conceptualized the study; AH and KH collected samples and compiled metadata; CK, SM, and SH performed analyses; CK, SH, AH, HGE, and KH interpreted the results and wrote the manuscript with input from SM and AK. All authors critically reviewed and contributed to the final version of the manuscript.

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Tables

Table 1. Genetic diversity per locus of Eurasian Lynx (*L. lynx*) in southern Finland (N = 282). Abbreviations: N_G = number of genotypes, N_A = number of different alleles per locus, H_O = observed heterozygosity, H_E = expected heterozygosity, F_{IS} = inbreeding coefficient.

Locus	N_G	N_A	Ho	He	F
Fca90	282	4	0.564	0.604	0.066
Fca723	282	7	0.603	0.582	-0.036
Fca082	282	5	0.706	0.732	0.036
Fca149	282	3	0.340	0.346	0.016
Fca567	282	6	0.748	0.755	0.009
Fca026	282	7	0.681	0.716	0.049
Fca031	281	8	0.758	0.798	0.051
Fca043	282	5	0.748	0.732	-0.023
Fca045	282	4	0.316	0.316	0.002
F115	282	12	0.773	0.826	0.064
Fca008	282	4	0.730	0.710	-0.029
Lc106	282	5	0.745	0.743	-0.002
Lc109	274	8	0.799	0.795	-0.005
Fca126	281	8	0.758	0.720	-0.053
Fca391	282	3	0.546	0.554	0.014
Fca275	281	8	0.804	0.786	-0.023
Fca293	281	3	0.591	0.594	0.006
Fca559	279	7	0.649	0.678	0.044
Lc110	282	5	0.582	0.594	0.022
Fca123	281	6	0.705	0.710	0.007
Fca077	282	6	0.525	0.615	0.147
Mean			0.651	0.662	0.017
SD			0.030	0.030	0.010

Table 2. Genetic diversity estimates for two genetic clusters and three geographical groups of Eurasian Lynx in southern Finland during 2003-2017. The analyzed groups are named according to genetic clusters (CL1, CL2) and the admixed groups: admixed females (ADXF) and admixed males (ADXM). Geographical regions are named numerically from west to east (GR1, GR2, and GR3). N = number of individuals. H_O (SE) = observed heterozygosity with standard error, H_E (SE) = expected heterozygosity with standard error, F_{IS} (SE) = inbreeding coefficients with standard error, F = females, M = males.

Group	N	H_O (SE)	H_E (SE)	F_{IS} (SE)
CL1F	29	0.632 (0.046)	0.602 (0.037)	-0.040 (0.028)
CL1M	46	0.627 (0.045)	0.615 (0.037)	-0.012 (0.034)
CL2F	52	0.651 (0.034)	0.654 (0.030)	0.004 (0.019)
CL2M	50	0.671 (0.022)	0.660 (0.024)	-0.023 (0.019)
ADXF	21	0.653 (0.040)	0.634 (0.032)	-0.032 (0.037)
ADXM	84	0.658 (0.032)	0.663 (0.029)	0.011 (0.018)
GR1F	40	0.630 (0.039)	0.613 (0.033)	-0.028 (0.026)
GR1M	54	0.673 (0.032)	0.658 (0.028)	-0.019 (0.014)
GR2F	22	0.641 (0.041)	0.647 (0.031)	0.020 (0.033)
GR2M	72	0.647 (0.028)	0.658 (0.027)	0.015 (0.016)
GR3F	40	0.664 (0.036)	0.659 (0.033)	-0,011 (0.022)
GR3M	54	0.643 (0.032)	0.662 (0.032)	0.021 (0.024)

Table 3. Analysis of Molecular variance (AMOVA) among genetic clusters and geographical regions for the Eurasian lynx in southern Finland 2003-2017.

STRUCTURE genetic clusters (CL1 = 75 individuals and CL2 = 102 individuals), and admixed (105 individuals). Likewise, an AMOVA was conducted for the three geographical regions (GR1, GR2, and GR3) with 94 individuals in each of the groups. To test for sex-specific patterns, the analysis was performed also for both sexes (F = females, M = males) separately.

	Sex	Between-sex variation (%)	Between-group variation (%)	F_{ST}	P
Genetic clusters 1, 2 and admixed	F	-	3.0	0.031	0.000
	M	-	3.0	0.026	0.000
	F+M	0	3.0	0.018	0.000
Genetic clusters 1 and 2	F	-	5.0	0.050	0.000
	M	-	6.0	0.056	0.000
	F+M	0	5.0	0.027	0.000
geographical regions GR1, GR2, GR3	F	-	2.0	0.022	0.000
	M	-	0	0.003	0.018
	F+M	0	1.0	0.010	0.000

Table 4 Genetic FSTAT statistic among genetic clusters and geographical regions for the Eurasian lynx in southern Finland 2003-2017. Values for females (N = 102) / males (N = 180) are given followed by the p-value. Abbreviations: mAIc = mean Assignment Indices corrected, vAIc = variance Assignment Indices corrected, F_{ST} = genetic differentiation, F_{IS} = inbreeding coefficient, REL = relatedness, H_O = observed heterozygosity, H_S = within-site gene diversity.

Entire range: Genetic clusters 1, 2 and admixed	**mAIc**	**vAIc**	**F_{ST}**	**F_{IS}**	**REL**	**H_0**	**H_S**
Females	0.948	15.009	0.031	-0.003	0.061	0.646	0.644
Males	-0.537	46.144	0.025	0.014	0.048	0.655	0.664
P-value	0.017	0.001	0.575	0.368	0.546	0.516	0.012

Geographical regions GR1, GR2, and GR3							
Females	0.805	12.244	0.022	0.003	0.042	0.646	0.648
Males	-0.456	22.265	0.003	0.017	0.006	0.654	0.665
P-value	0.005	0.001	0.001	0.275	0.001	0.246	0.010

Figures

Fig 1. The study area for Eurasian lynx in Southern Finland. Spatial distribution of the two genetic clusters for both male and female Eurasian lynx (N_{male}= 180; N_{female}= 102) as well as admixed individuals in black. Black boxes indicate the three geographical regions used for the LOCPRIOR function in STRUCTURE and as an alternative grouping for summary statistics studied.

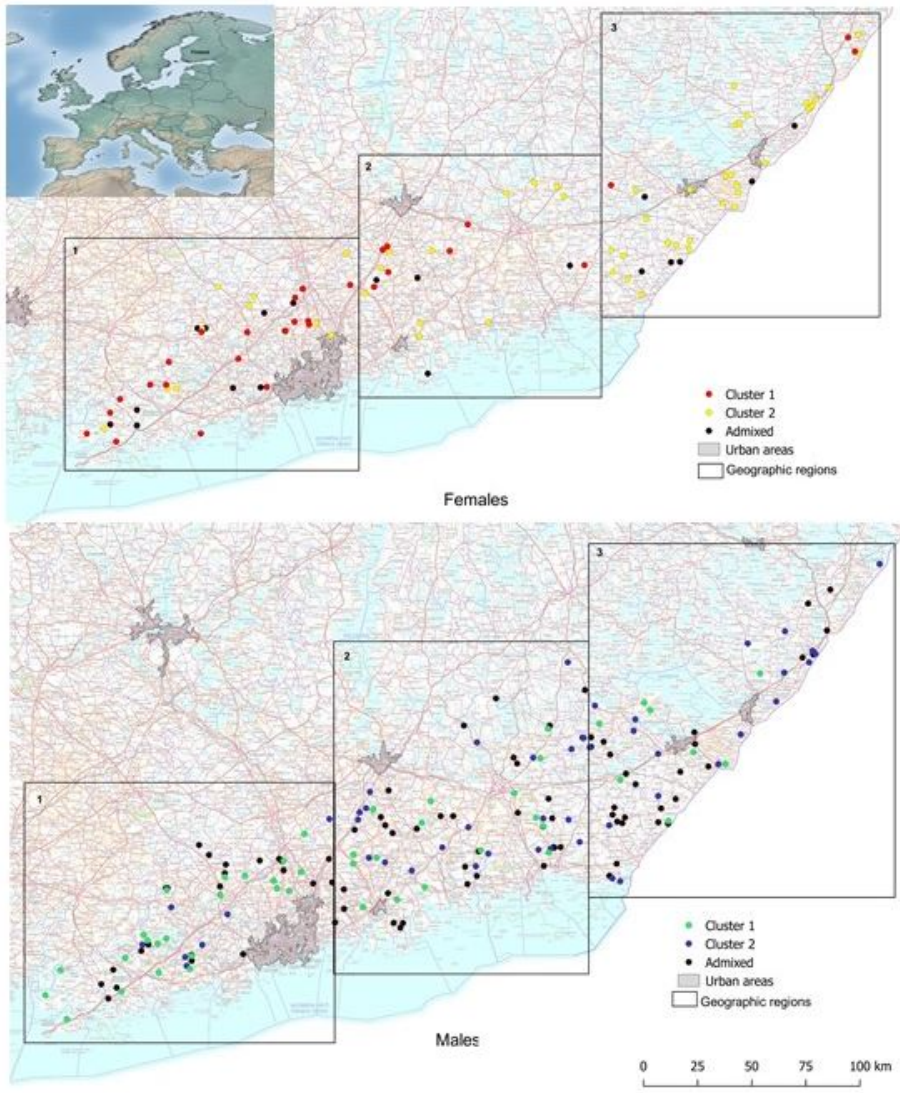


Fig 2. Spatial genetic structure identified by Bayesian cluster assignment analysis with STRUCTURE for Eurasian lynx in southern Finland. Fig 2A-C show the DeltaK plots for 2A) the combined data set, 2B) females, and 2C) males, respectively. Fig 2D-F display the mean of estimated log-likelihood values for 2D) the combined data set, 2E) females, and 2F) males. Fig 2G-J. CLUMPAK-averaged Bayesian clustering (STRUCTURE) plots showing posterior probabilities of Lynx lynx individual genotypes (as bars) assigned to each genetic

cluster based on STR data for K = 2 - 3. Individuals are sorted by geography from west to east in STRUCTURE bar plots. Fig 2G) sexes combined (N = 282), 2H) females (N = 102), and 2I) males (N = 180). In addition, Fig 2J) shows the bar plot for females across three geographical regions as retrieved from a STRUCTURE run with the LOCPRIOR option.

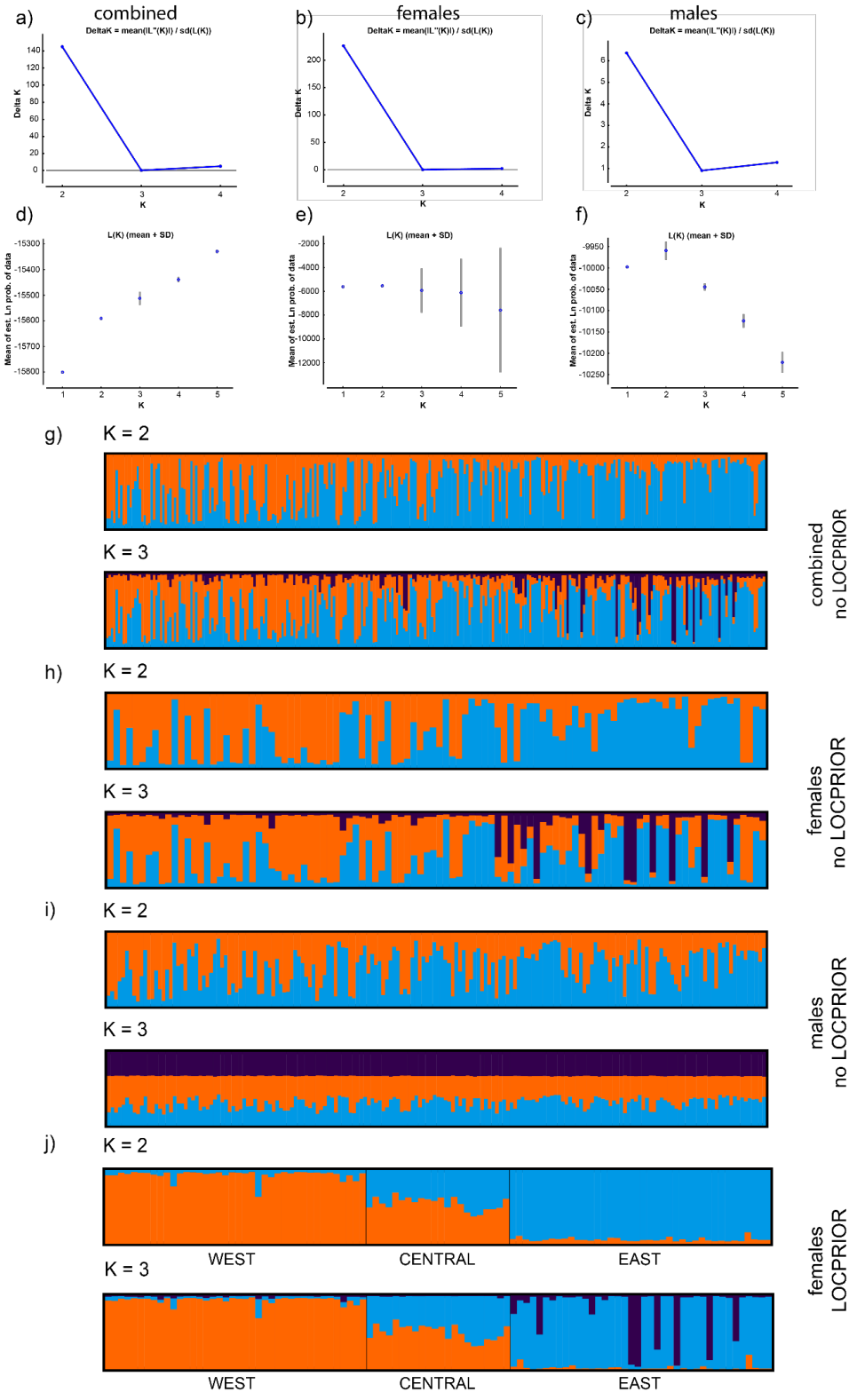

a)
combined
b)
females
c)
males
DeltaK = mean(|L''(K)|) / sd(L(K))
Delta K
K
d)
e)
f)
L(K) (mean + SD)
Mean of est. Ln prob. of data
g)
K = 2
K = 3
combined
no LOCPRIOR
h)
females
no LOCPRIOR
i)
males
no LOCPRIOR
j)
WEST
CENTRAL
EAST
females
LOCPRIOR

Fig 3. Spatial autocorrelation within geographic distance classes for genetic clusters and geographical groups for the Eurasian lynx in southern Finland 2003-2017. Fig 3A) Combined spatial structure analysis for females in both genetic clusters and the admixed individuals. 3B) Combined spatial structure analysis for males in both genetic clusters and the admixed individuals. 3C) Spatial autocorrelation for females in both genetic clusters and admixed individuals. 3D) Spatial autocorrelation for females in both genetic clusters and admixed individuals. genetic cluster 1 = blue line, genetic cluster 2 = orange line, admixed individuals = grey line. F = females, M = males. 3E) Combined spatial structure analysis for females based on geographical regions. 3F) Combined spatial structure analysis for males based on geographical regions. 3G) Spatial autocorrelation for females in the three geographical regions. 3H) Spatial autocorrelation for males in the three geographical regions. Geographical region 1 = blue line, geographical region 2 = orange line, and geographical region 3 = grey line. The 95% confidence interval for the Null hypothesis of random distribution is given as a dashed line, the bootstrap errors are displayed as whiskers.

Figure 4.

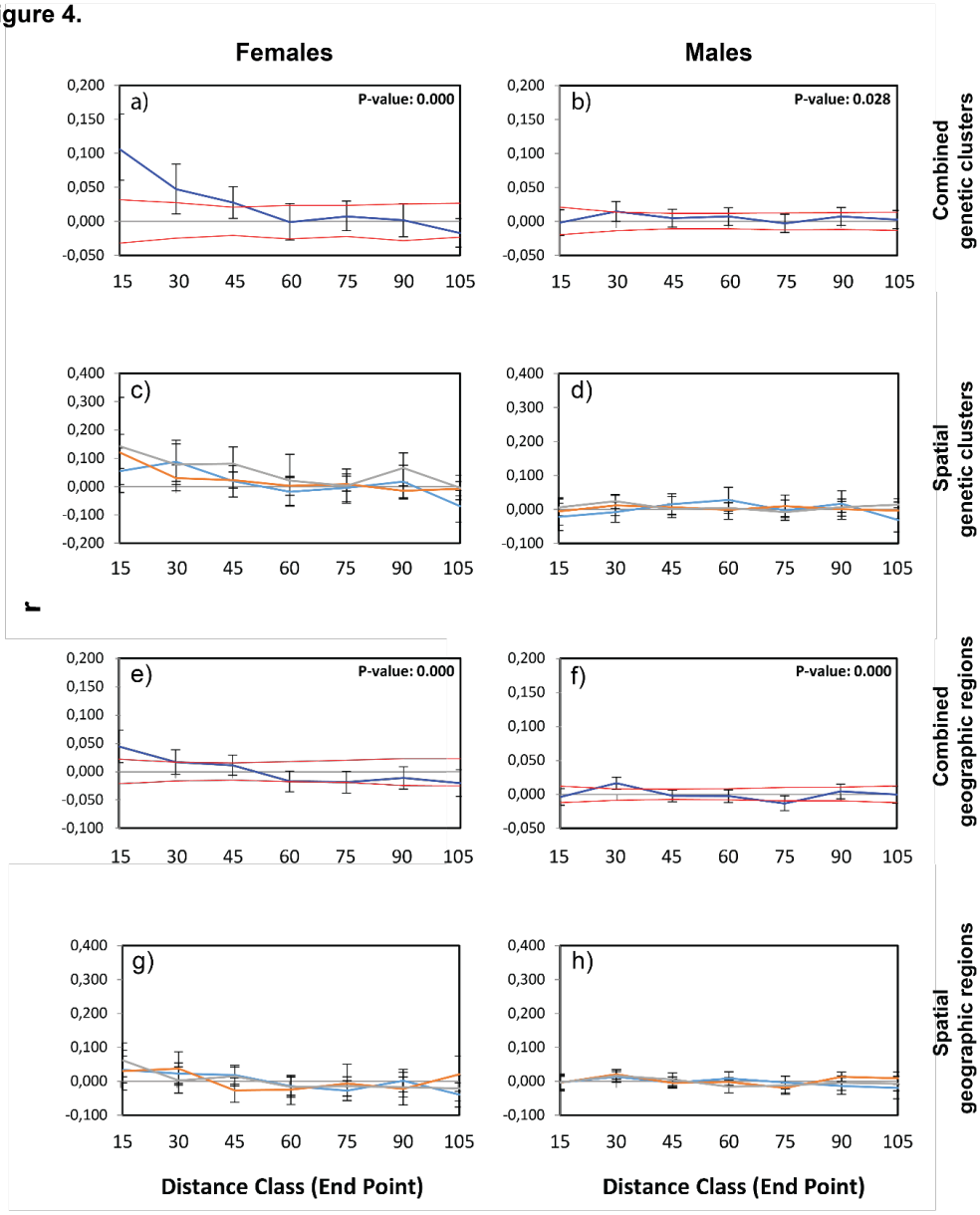


Fig 4. Frequency distribution and mean of corrected genetic assignment indices (AIc) for lynx males and females of Eurasian lynx in southern Finland 2003-2017. Fig 4A) Frequency distribution of AIc (corrected Assignment Index) values in the entire data set after taking CL3 females out of analysis. Data for males shown in black bars and data for females shown in white bars. Fig 4B) Mean AIc values in males and females.

Fig 4A)

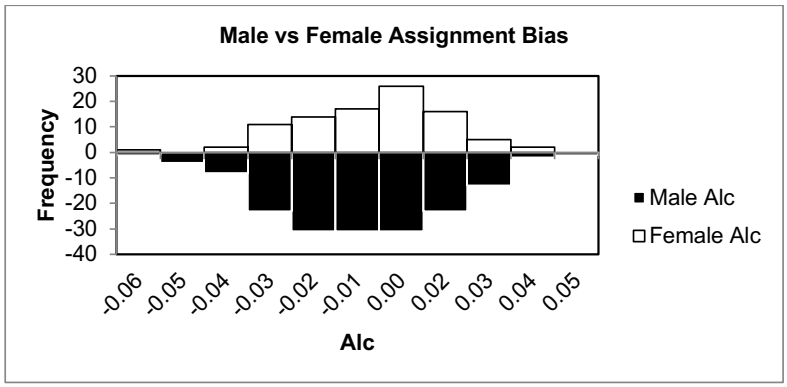


Fig 4B)

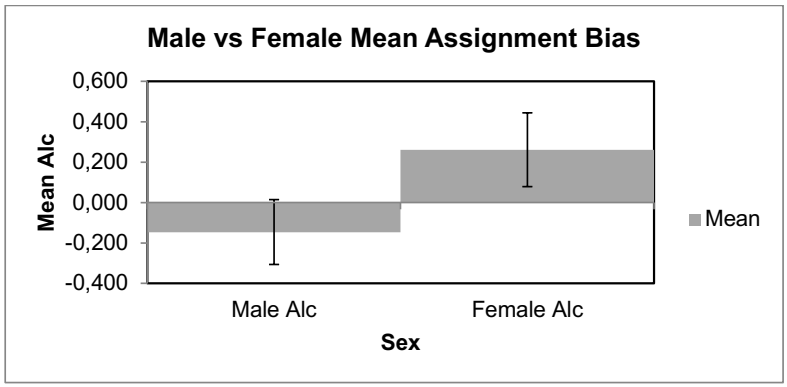


Fig 5. Comparison of relatedness estimates among genetic clusters (A) and geographical regions (B) for the Eurasian lynx in southern Finland 2003-2017. Abbreviations: CL1F = cluster 1 females, CL2F = cluster 2 females, ADXF = admixed females, CL1M = cluster 1 males, CL2 = cluster 2 males, ADXM = admixed males. GR1F = geographical region 1, GR2 = geographical region 2, GR3 = geographical region 3. The upper (U) and lower (L) boundaries for the 95% confidence interval for the null hypothesis of random distribution is given as red lines, the bootstrap errors are displayed as whiskers.

Fig 5A)

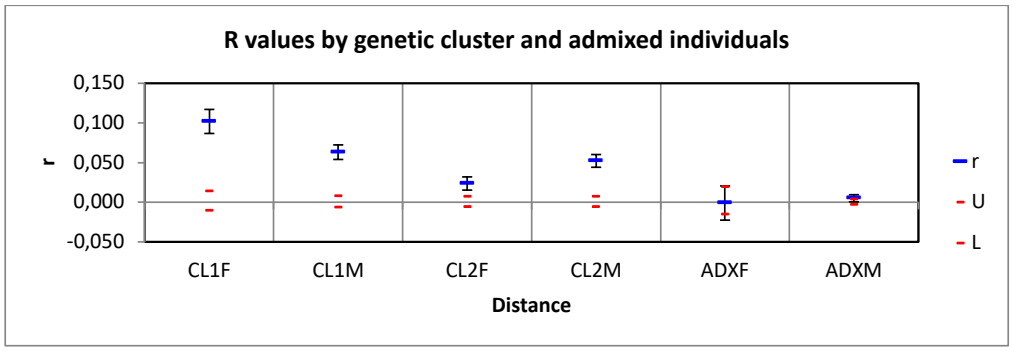
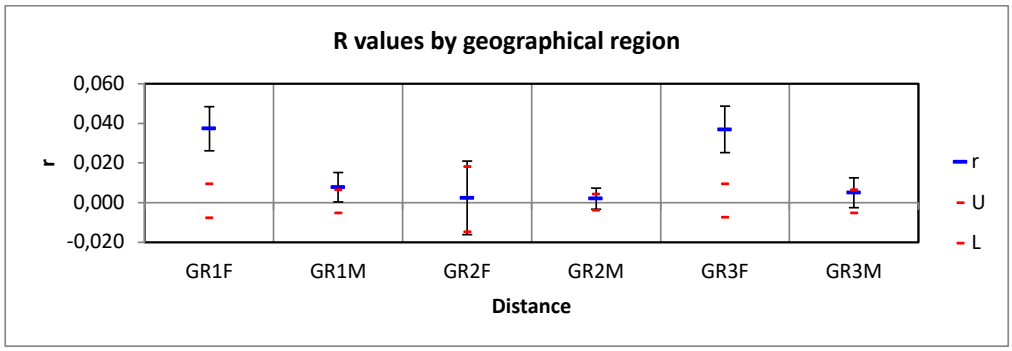

R values by genetic cluster and admixed individuals
0,150
0,100
0,050
0,000
-0,050
r
CL1F
CL1M
CL2F
CL2M
ADXF
ADXM
Distance
r
U
L

Fig 5B)


R values by geographical region
0,060
0,040
0,020
0,000
-0,020
r
GR1F
GR1M
GR2F
GR2M
GR3F
GR3M
Distance
r
U
L

III

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ORIGINAL RESEARCH

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Genetic evidence of female kin clusters in a continuous population of a solitary carnivore, the Eurasian lynx

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Abstract

Large terrestrial carnivores can sometimes display strong family bonds affecting the spatial distribution of related individuals. We studied the spatial genetic relatedness and family structure of female Eurasian lynx, continuously distributed in southern Finland. We hypothesized that closely related females form matrilineal assemblages, clustering together with relatives living in the neighboring areas. We evaluated this hypothesis using tissue samples of 133 legally harvested female lynx (from year 2007 to 2015), genotyped with 23 microsatellite markers, and tested for possible spatial genetic family structure using a combination of Bayesian clustering, spatial autocorrelation, and forensic genetic parentage analysis. The study population had three potential family genetic clusters, with a high degree of admixture and geographic overlap, and showed a weak but significant negative relationship between pairwise genetic and geographic distance. Moreover, parentage analysis indicated that 64% of the females had one or more close relatives (sister, mother, or daughter) within the study population. Individuals identified as close kin consistently assigned to the same putative family genetic cluster. They also were sampled closer geographically than females on average, although variation was large. Our results support the possibility that Eurasian lynx forms matrilineal assemblages, and comparisons with males are now required to further assess this hypothesis.

KEYWORDS
genetic relatedness, kinship, *Lynx lynx*, matrilineal assemblage

1 | INTRODUCTION

Large terrestrial carnivores with high rates of dispersal and long-distance movement usually also have high rates of gene flow among populations if dispersing individuals succeed to reproduce (Wayne & Koepfli, 1996). Even with highly mobile species, discontinuous habitat and anthropogenic-associated barriers, such as major roads, monoculture, and human-caused mortality, may constrain dispersal and reduce population densities (Loxterman, 2011; Sinclair et al., 2001; Walker, Harveson, Pittman, Tewes, & Honeycutt, 2000; Woodroffe, 2000). Accordingly, fragmentation and genetic drift may cause genetic substructuring in the population, for example gray wolf (*Canis lupus*) and Florida black bear (*Ursus americanus floridanus*) (e.g., Dixon et al., 2007; Vilà et al., 1999; Wayne, Lehman, Allard, & Honeycutt, 1992). In continuous populations, unhampered by fragmentation or isolation, dispersal and gene flow can be assumed to be less affected by geographic barriers, but rather by social, ecological, and evolutionary constraints (Rueness et al., 2003). This creates

possibilities for assessment of social organization as the cause of local family genetic structuring, independent of the potentially confounding effects of population fragmentation and geographic isolation. In recent decades, several large carnivore species have recolonized parts of their former distribution range and in some areas regained a continuous distribution over large unfragmented landscapes (Chapron et al., 2014).

Due to their different social organization, group-living and solitary large carnivores may differ in the frequency and strength in which they form long-lasting family bonds and, more generally, in the rate in which they interact with other individuals during their lifetime. Little is known about the association between social and family genetic structure, especially in solitary large carnivores. Behavioral studies of leopard (*Panthera pardalis*), puma (*Puma concolor*), and tiger (*Panthera tigris*) have revealed that also solitary species can exhibit kin clusters (Elbroch, Quigley, & Caragiulo, 2015; Fattebert et al., 2016; Goodrich et al., 2010; Logan & Sweanor, 2002), but the family genetic structure is less studied.

The Eurasian lynx (*Lynx lynx*) is a solitary predator with a social organization based on territoriality. The species has one of the most widespread distributions of the currently living felids (Breitenmoser et al., 2015). The lynx populations inhabiting Europe differ in their population history and degree of habitat discontinuity (von Arx, Breitenmoser-Wuersten, Zimmermann, & Breitenmoser, 2004), but in many parts, their populations are highly fragmented (Kaczensky et al., 2013). Thus, it is often challenging to determine the influence of social, ecological, and evolutionary constraints on genetic relatedness and family genetic structure, as this ideally requires high-resolution genetic data from a continuous and unfragmented population. For the lynx family, studies on kin clusters and philopatry have given inconclusive results. A link between kin structure and dispersal has been found for bobcat (*Lynx rufus*) (Croteau, Heist, & Nielsen, 2010; Janečka et al., 2006), but not for Canada lynx (*Lynx canadensis*) (Campbell & Strobeck, 2006). In Sweden, telemetry studies showed that about one third of Eurasian lynx female offspring remained philopatric (Samelius et al., 2011), indicating the potential for geographic clustering of female relatives. Schmidt, Davoli, Kowalczyk, and Ettore (2016) found an insignificant pairwise geographic genetic distance relationship in a small isolated population of lynx in Białowieza, Poland, but it remains unknown if these results can also be found in other, continuous populations on larger scales. In Latvia, a genetic approach was used to identify the number and location of family groups based on parent–offspring relationships, but details on family genetic structure were not provided (Bagrade et al., 2016).

In this study, we have investigated the genetic relatedness and family structure of Eurasian lynx females in southern Finland. The Eurasian lynx is the only felid species in Finland and the population has shown a substantial population recovery and range expansion during late 1990s and early 21st century (Chapron et al., 2014). The population estimate is based on family group counts, method modified from that in use in Scandinavia (Andrén et al., 2002; Linnell et al., 2007), and has increased from 1,100 to 2,700 adult individuals during years 2007 to 2015 (Luke, 2017), corresponding to an average yearly increase of 12% (Lambda = $(2{,}700/1{,}100)^{1/8}$ = 1.12). Distribution area covers whole of Finland, with highest densities in south and central parts of the country (details in: Holmala, 2013)). In this continuously distributed population, we analyzed the genetic family structure using 23 autosomal microsatellite markers and tested for possible spatial genetic family structure using a combination of Bayesian clustering, spatial autocorrelation, and forensic genetic parentage analysis. This allowed us to study how genetic relatedness and family structure are organized in space in female lynx unconstrained by low population size, isolation or fragmentation.

2 | MATERIALS AND METHODS

2.1 | Study area

Our study area encompasses two game management districts in the southeastern and eastern parts of Finland, which have a total area of 22,936 km^2 (Figure 1). About 70% of the terrestrial area is covered by forest, where pine, spruce, and birch are the most common trees. Roughly 10% of the region is covered by lakes. Together with mires,

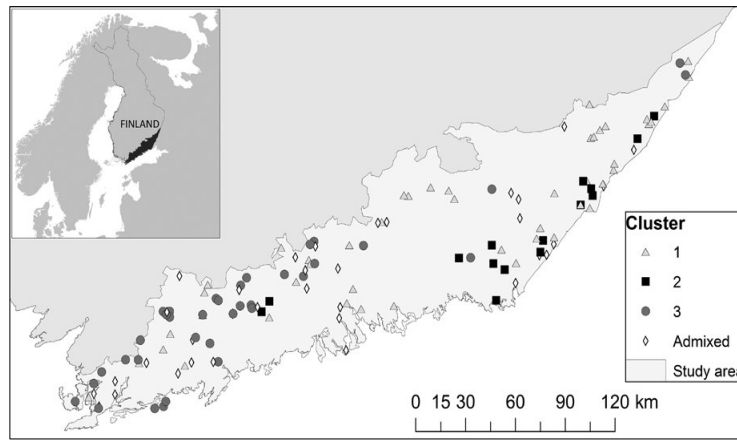

FIGURE 1 Location of the study area, locations of the Eurasian lynx female tissue samples from years 2007 to 2015, and their identified clusters in southern Finland. Symbols denote the genetic cluster each sample was assigned to cluster 1 = gray triangle; cluster 2 = black square; cluster 3 = dark gray circle; A (admixed) = hollow diamond. *N* = 132

farm land, and urban areas, these two regions form a heterogeneous mosaic of different land uses.

2.2 | Sampling and DNA extraction

We collected tissue samples from 133 female lynx that had either died naturally (4), in traffic accidents (15) or hunted legally (114) under derogation licenses (granted by the Finnish Wildlife Agency and the Ministry of Forestry and Agriculture) during 2007 to 2015 in Finland (Figure 1). Lynx carcasses were sent to the Taivalkoski Research Station of the Natural Resources Institute Finland (Luke), where the tissue samples were collected and frozen down immediately. Ages of the individuals were determined from cementum annuli analysis of tooth samples by the Matson Laboratory, Milltown, Montana (Matson, 1981).

DNA was extracted from tissue samples with the DNeasy Tissue kit (Qiagen) in the laboratory of Norwegian Institute of Bioeconomy Research, NIBIO—Svanhovd. Samples were analyzed with 23 microsatellite (Short-tandem repeats; STRs) markers, 20 of which were originally identified for the domestic cat (*Felis catus*; Menotti-Raymond et al., 1999), whereas the remaining three markers were developed specifically for the Canada lynx (*L. canadensis*) (Lc106, Lc109, Lc110; Carmichael, Clark, & Strobeck, 2000). Additionally, gender identification was performed using the markers on the zinc finger region on the X- and Y-chromosome developed for felids (Pilgrim, McKelvey, Riddle, & Schwartz, 2005). The markers were chosen based on their use in several population genetic studies about lynx in northern Europe (e.g., Hellborg et al., 2002; Rueness et al., 2003; Schmidt, Kowalczyk, Ozolins, Männil, & Fickel, 2009; Ratkiewicz et al., 2014; Rueness, Naidenko, Trosvik, & Stenseth, 2014). Markers were combined into seven multiplex sets (Table 1), and amplification was performed with 1 µl template DNA in a 10 µl PCR containing 5.0 µl 2x Multiplex PCR MasterMix (Qiagen), 1.0 µl Primermix, 0.05 µl BSA (NEB), and 2.95 µl ddH2O. Primer concentration and composition of the different multiplex sets are given in Table 1. The PCR thermal profile was as follows: 10 min at 95°C followed by 29 cycles of 30 s at 94°C, 30 s at 56/57/58/59°C (annealing temperature varied according to multiplex set, see Table 1), 1 min at 72°C, with a final elongation step of 45 min at 72°C. Samples were analyzed on an ABI PRISM 3730 sequencer, and genotyping was performed with GeneMapper v4.1 (Applied Biosystems). Tissue samples were generally analyzed once; however, in order to assess genotyping reliability, 10% of the samples chosen randomly were analyzed a second time.

TABLE 1 23 different STR markers and the XY-test used in the genetic analysis. Markers are ordered according to the multiplex set they were assigned to, including the respective annealing temperatures (AT) and fluorescent label (Flag). Final concentration of the respective markers was the same for both forward and reverse primer

Multiplex set	AT (°C)	Marker	Flag	Concentration (µM)
1	58	Fca090	FAM	0.2 µM
		Fca149	VIC	0.1 µM
		Fca723	FAM	0.2 µM
		Fca082	FAM	0.1 µM
2	58	Fca559	NED	0.3 µM
		Fca275	FAM	0.2 µM
		Fca293	FAM	0.2 µM
3	58	Lc110	FAM	0.1 µM
		Fca123	FAM	0.3 µM
		Fca001	FAM	0.4 µM
4	59	Fca567	FAM	0.2 µM
		Fca026	FAM	0.2 µM
		Fca078	FAM	0.3 µM
		Fca031	FAM	0.3 µM
5	58	Fca043	FAM	0.15 µM
		Fca045	NED	0.15 µM
		Fca008	FAM	0.15 µM
		F115	FAM	0.4 µM
6	57	Lc106	FAM	0.5 µM
		Fca126	NED	0.1 µM
		Lc109	FAN	0.5 µM
		Fca391	NED	0.1 µM
7	56	Fca077	FAM	0.2 µM
		XY zinc finger	VIC	0.05 µM

2.3 | Family genetic structure, spatial autocorrelation, and genetic parentage analysis

We used STRUCTURE v.2.3.4 (Falush, Stephens, & Pritchard, 2003; Pritchard, Stephens, & Donnelly, 2000) to test for indications of family genetic clustering among the females. We set the maximum number of populations to 10 (K = 10) with 10 independent runs for each K and assuming population admixture and correlated allele frequencies. Burn-in period was 100,000 Markov-Chain-Monte-Carlo (MCMC) iterations, with a subsequent sampling of 1,000,000 MCMC iterations. We processed the results with Structure Harvester (Earl & von Holdt, 2012), which implements the ad hoc approach of Evanno, Regnaut, and Goudet (2005), and determined the number of putative family genetic clusters, using a membership value of $q \geq 0.7$ as a threshold value (Kopatz et al., 2014; Pelletier, Obbard, Mills, Howe, & Burrows, 2012). Based on cluster assignment, we performed a factorial correspondence analysis (FCA) among all genotypes, and calculated the number of alleles, observed and expected heterozygosity using GenAlEx 6.5 and inbreeding coefficient using Genetix 4.05.2 (Belkhir, Borsa, Chikhi, Raufaste, & Bonhomme, 1996–2004).

We performed a spatial autocorrelation analysis using GenAlEx 6.501 (Peakall & Smouse 2006; Peakall & Smouse 2012) to examine the relationship between genetic and spatial distance among each pair of female lynx. In the analysis, the family genetic structuring indicated by STRUCTURE was taken into account by using a multipopulation approach. To strike the balance between sample size

TABLE 2 Genetic variation in the studied female lynx population from southern Finland (N = 132) during the years from 2007 to 2015. Expected (H_E) and observed (H_O) heterozygosity, number of different alleles (N_A), and inbreeding values (F_{IS}) calculated for the 23 short-tandem repeats. Loci deviating significantly from Hardy–Weinberg equilibrium are highlighted by bold F_{IS} values

Locus	N_A	H_E	H_O	F_{IS}
Fca90	4	0.57	0.55	0.044
Fca723	7	0.54	0.58	−0.063
Fca082	5	0.72	0.72	0.012
Fca149	3	0.32	0.30	0.051
Fca567	5	0.77	0.82	−0.069
Fca026	7	0.72	0.72	−0.006
Fca078	5	0.77	0.79	−0.023
Fca031	8	0.80	0.78	0.021
Fca043	5	0.74	0.80	−0.074
Fca045	3	0.30	0.29	0.040
F115	10	0.83	0.79	0.048
Fca008	4	0.72	0.74	−0.028
Lc106	5	0.74	0.74	0.008
Lc109	8	0.78	0.79	−0.011
Fca126	7	0.74	0.77	−0.029
Fca391	3	0.55	0.50	0.090
Fca275	8	0.80	0.77	0.045
Fca293	3	0.60	0.61	−0.006
Fca559	5	0.67	0.61	**0.098**
Lc110	5	0.60	0.55	0.074
Fca123	6	0.71	0.70	0.013
Fca001	8	0.70	0.69	0.010
Fca077	4	0.58	0.52	0.108
Mean	5.6	0.66	0.66	0.012
SD	1.9	0.14	0.15	

and spatial resolution, a distance class of 15 km was chosen which corresponds roughly to the largest known female home ranges (i.e., radius) in southern Finland based on telemetry data (K. Holmala unpublished). For each distance class, statistical significance was inferred if the 95% CI around r (the average relatedness) did not contain 0, and if r exceeded the 95% CI around the null hypothesis of r = 0, that is no spatial structure.

We used the program Familias 3.1.9.5 (Egeland & Mostad, 2000; Kling, Tillmar, & Egeland, 2014) to reconstruct parenthood and sib ship from the microsatellite data by calculating likelihood ratios (LRs) for mother–offspring and sibling relationship. The Familias software is used worldwide by forensic laboratories and has been applied to numerous cases, for example resolving family relations and individual identification after disasters. Familias is programmed to recognize several different relationships and makes a pairwise comparison with all individual genotypes against each other and calculates an LR for each relationship. This makes an objective way to distinguish between the most likely relationships. The LR represents the probability of hypothesis one (candidate female is the true mother) divided by the probability of hypothesis two (candidate female is unrelated to the offspring in question) (Marshall, Slate, Kruuk, & Pemberton, 1998).

Based on the a priori probability of being related or unrelated equals to 0.5, the LR shows which relationship is more likely than others. This means a LR of 20 from the genetic analysis corresponds to a 95% probability for relatedness and would be considered as significant support for the relationship in question, while a LR value above 100 would be considered as highly significant support (99% probability of relatedness). Calculating family relationships requires allele frequency data of the population in question and a kinship correction. These we deduced from the data of this study. The Familias software is used worldwide by forensic laboratories and has been applied to numerous cases, for example resolving family relations and individual identification after disasters. In a second step, we used the pairwise geographic distances between death locations of each pair of individuals to calculate the mean and median distance between female parent–offspring, siblings, and all individuals. We also expected that individuals within pairs identified as close relatives would assign to the same family cluster, which was checked against the STRUCTURE results.

3 | RESULTS

3.1 | Microsatellite genotyping

We obtained a full genetic profile of 23 STRs for 132 samples (N = 133). One sample did not contain enough DNA for successful amplification with the employed STR markers. All of the samples genotyped successfully received a unique identity. For 13 samples, analysis of one or more markers had to be repeated due to amplification failure in the first round, when three tissue samples showed no amplification success in one or two markers.

3.2 | Genetic diversity

Genetic diversity was relatively high, and expected heterozygosity and observed heterozygosity were almost identical, both averaging 0.66 across all markers (Table 2). Number of alleles varied between 3 and 10 across all 23 markers. There was no evidence of inbreeding in the population, with F_{IS} values not deviating significantly from 0 and averaging 0.012 across all markers and individuals. Tests for HWE followed by Bonferroni correction showed no significant deviation from Hardy–Weinberg equilibrium for any of the markers, except for the marker Fca 559.

3.3 | Fine-scale genetic family structure

Based on the estimated mean likelihood values and Evanno's ΔK (Figure 2b), the STRUCTURE analysis grouped the 132 female lynx individuals in three potential family genetic clusters, which displayed only a slight shift in distribution across the study area

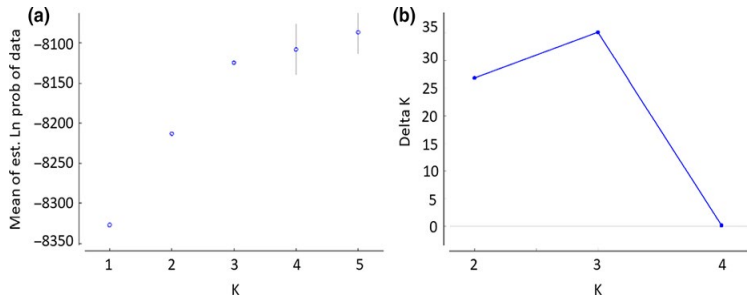

FIGURE 2 (a) Mean of estimated log-likelihood values, and (b) rate of log-likelihood values for 132 Eurasian lynx females for different number of clusters from the software STRUCTURE (Pritchard et al., 2000) postprocessed with Evanno's approach (Evanno et al., 2005)

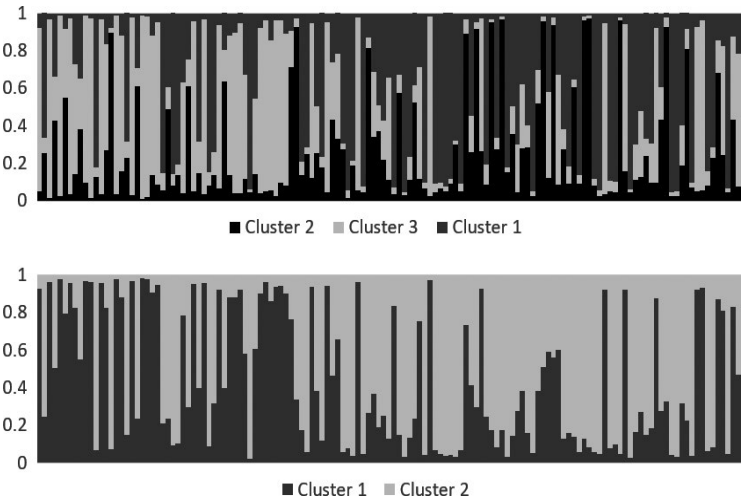

FIGURE 3 Results from the Bayesian cluster assignment analysis with STRUCTURE of 132 female lynx from southern Finland from 2007 to 2015. Upper panel: model with three clusters; lower panel: model with two clusters. Samples are grouped according to their harvest location and sorted by longitude from west (left) to east (right). The *y*-axis indicates the membership coefficient *q*, that is the likelihood of belonging to a particular cluster. Each bar represents one individual and the length of each section of one bar corresponds to the *q* value for the respective cluster

(Figure 1). Thirty-four of 132 lynx could not be assigned to any of the three detected clusters unambiguously. To some extent, ΔK supported also an alternative model with only two spatially overlapping family genetic clusters (Figures 2 and 3). Also with this model, the identified clusters displayed only a slight shift in distribution across the landscape (Figure 3), somewhat reminiscent of a geographic–genetic distance relationship. The existence of three potential family genetic clusters across the study area was also supported by an independent FCA, showing three groups for the assigned genotypes and admixed genotypes located among the clusters (Figure 4).

Consistent with the observed high spatial overlap among the potential family genetic clusters, there was a relatively flat but still highly significant negative correlation between genetic and geographic distance among the 132 female lynx (Figure 5). The average relatedness r of pairs of females was low, but positive and significantly above what one would expect with a random distribution of individuals in all distance classes up to 45 km distance. For the distance class of 60 km, the average relatedness index sunk close to zero, but increased again around 75 km until again falling within the bounds of the 95% CI at the distance classes of 90 km and more.

3.4 | Genetic parentage analysis

Parentage analyses using the program Familias suggested close, genetic relationships between many different pairs of female lynx: parent–offspring, sibling or half-sibling, and these pairs of close kin were found both within all three genetic clusters and within the group of admixed individuals (Tables 3–5). Individuals within pairs of close kin consistently assigned to the same genetic cluster, except in some instances where one of the individuals in a pair was admixed. Among individuals having at least 2-year difference in age, 32 pairs (47 individuals) were identified as likely mother and daughter by significant support that is a LR above 20 (Table 3). Of these, 97% showed 99% probability of relatedness. Furthermore, 64 pairs (79 individuals) showed LR >20 for being siblings and of these, 68% showed 99% probability of relatedness. In total, 26 pairs (42 individuals) showed LR >20 in both of the analyses above and thus identified as both likely mother–daughter and likely sisters (Table 5). In all of these cases, LR was higher for mother–daughter than for sister, which was also supported by a relatively large age difference in most cases. Assuming that all of these were mother–daughter relationships, the total number of significant sister relationships would be reduced to 38 (57 individuals;

Table 4), and the total percentage of individuals with at least one close kin (mother–daughter and sister–sister) would be 63.6% (84 ind.), with 36.4% (48 ind.) being unrelated to all others. The median geographic distance between identified mother–offspring pairs was 36.8 km (mean distance 112.6 km; mean age difference 5.3 years) and between siblings 55.6 km (mean distance 108.6 km; mean age difference 2.5 years). In comparison, the pairwise median geographic distance between all females was 228.6 km (mean distance 262.8 km; mean age difference 3.03 years; Figure 6).

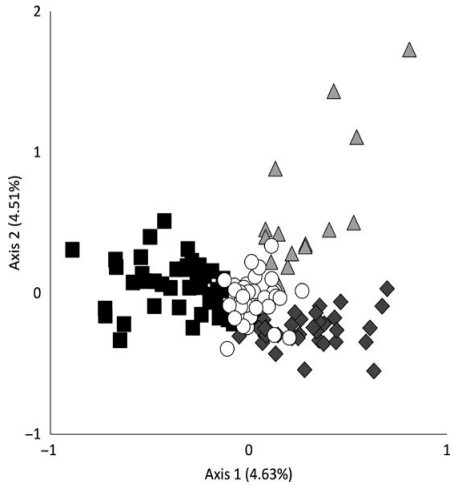

FIGURE 4 Visualization of the factorial correspondence analysis (FCA) for female lynx genotypes sampled in southern Finland in the time period from 2007 to 2015. Different colors represent the clusters identified by the Bayesian clustering approach: cluster 1 (black squares), cluster 2 (light gray triangles), cluster 3 (gray diamonds), and admixed individuals with a cluster membership value $q < 0.7$ (white circles)

4 | DISCUSSION

Using three different genetic approaches, we found support for family genetic structure among females of a solitary carnivore, the Eurasian lynx. The potential family groups were represented by spatially overlapping clusters and a flat but negative pairwise genetic–geographic distance relationship. Furthermore, we found evidence that within each of the putative family genetic clusters, there were several different sibling or mother–daughter pairs. Thus, our results suggest that in a spatially unrestricted population of Eurasian lynx, closely related females tend to cluster together geographically, in agreement with the hypothesis that they may form matrilineal assemblages when not constrained by low density and population fragmentation.

Our estimates of genetic diversity of Eurasian lynx from southern Finland are among the highest. In a review, Schmidt, Ratkiewicz, and Konopiński (2011) concluded that the Eurasian lynx has low to moderate genetic variability. Genetic variation was lowest in Scandinavia and, overall, populations displayed high differentiation and fragmentation (Schmidt et al., 2011). The higher variability for Finnish lynx found in our study may be explained by the larger population size, following successful population recovery and a viable contact through exchange with the larger continuous Russian population (Ratkiewicz et al., 2014; Rueness et al., 2014).

Dispersal has been identified as one of the key elements affecting the genetic structuring of populations. Dispersal barriers are clearly not the reason for the observed family genetic clustering, as the clusters were highly intermixed and not spatially separated. Also, no effective geographic or anthropogenic-associated barriers for lynx are known from southern Finland. Moreover, the fine-scale genetic clustering found in our study is on a much smaller spatial scale than would normally be considered relevant for studying genetic subpopulations, stretching only to the similar extent as the diameter of several lynx home ranges (K. Holmala unpublished, Linnell et al., 2007), and with siblings or mother–daughter pairs consistently occurring within clusters. Furthermore, Eurasian lynx

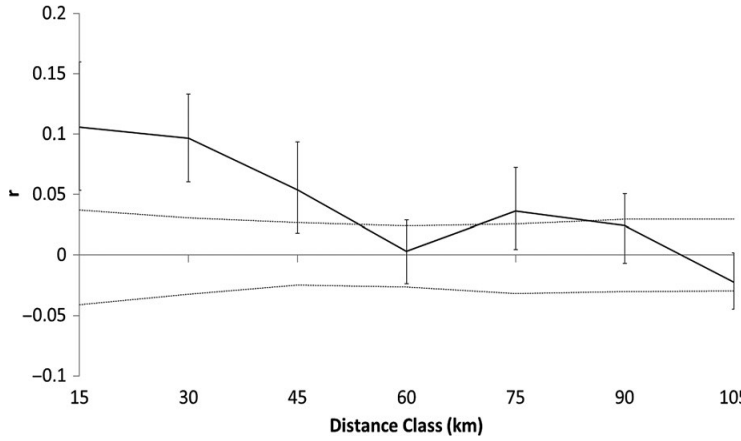

FIGURE 5 Results of the spatial autocorrelation analysis, that is combined correlation between genetic and spatial distance with GenAlEx 6.501 of lynx samples from southern Finland in the time period 2005 to 2015. The estimated relatedness coefficient (*r*; *y*-axis) for each distance class (*x*-axis) is given as a solid line. The 95% confidence interval for the Null hypothesis of random distribution is given as a dashed line, the bootstrap errors are displayed as whiskers. $N = 132$

TABLE 3 Recognized 32 Mother–offspring relationships (47 individuals) in a lynx population in southern Finland during the years 2007–2015. The individuals have at least a 2-year age difference. The likelihood ratios (LR) above 20 were sorted from the highest to the lowest. Individual identification contains ID-number, year of birth, year of death, and genetic cluster (1, 2, 3, and A = admixed)

Individual 1	Individual 2	Age difference	LR	Distance (km)
FiLL060_2008_2012_2	FiLL075_2000_2013_2	8	4,556,980.00	11.99
FiLL004_2005_2007_1	FiLL041_2000_2011_1	5	2,335,580.00	35.17
FiLL044_2011_2012_3	FiLL086_2006_2013_3	5	650,720.00	13.49
FiLL071_2009_2012_2	FiLL075_2001_2013_2	9	470,696.00	13.32
FiLL084_2013_2013_3	FiLL086_2006_2013_3	7	232,597.00	3.49
FiLL091_2012_2014_1	FiLL130_2005_2015_1	8	151,655.00	278.58
FiLL007_1997_2008_1	FiLL053_2007_2012_1	9	144,159.00	59.38
FiLL007_1997_2008_1	FiLL099_2001_2014_1	3	110,972.00	17.65
FiLL051_2009_2012_1	FiLL058_2012_2012_1	2	106,152.00	19.24
FiLL060_2008_2012_2	FiLL112_2011_2014_2	3	101,015.00	534.65
FiLL049_2004_2012_3	FiLL074_2011_2013_3	6	63,841.50	43.93
FiLL013_2004_2008_A	FiLL045_2003_2012_A	2	21,391.80	69.01
FiLL030_2008_2011_2	FiLL067_2006_2012_2	2	19,333.80	103.98
FiLL091_2012_2014_1	FiLL127_2014_2014_1	2	13,378.30	272.93
FiLL034_2010_2011_2	FiLL067_2006_2012_2	4	11,748.10	6.74
FiLL001_2004_2006_A	FiLL031_2001_2011_1	4	7,866.37	69.92
FiLL101_2013_2014_3	FiLL113_2010_2015_3	4	5,882.27	460.77
FiLL016_2006_2008_3	FiLL113_2010_2015_3	3	3,574.93	421.46
FiLL011_1994_2007_A	FiLL017_2007_2009_A	12	3,547.81	9.38
FiLL004_2005_2007_1	FiLL050_2011_2012_1	5	3,531.88	56.99
FiLL045_2002_2012_A	FiLL059_2012_2012_1	9	3,259.75	1.51
FiLL049_2004_2012_3	FiLL088_2011_2013_3	7	2,382.41	33.71
FiLL040_2009_2011_A	FiLL076_2012_2013_A	2	2,245.33	62.05
FiLL008_2005_2008_1	FiLL118_2014_2014_1	9	1,712.52	19.45
FiLL046_2002_2012_A	FiLL125_2012_2015_A	9	1,662.39	38.07
FiLL063_2011_2012_3	FiLL101_2014_2014_3	2	1,008.62	35.47
FiLL066_2012_2012_A	FiLL068_2008_2012_A	4	961.77	9.39
FiLL063_2011_2012_3	FiLL113_2010_2015_3	2	869.96	426.24
FiLL018_2008_2009_1	FiLL041_2000_2011_1	8	766.41	30.35
FiLL021_2008_2010_A	FiLL046_2003_2012_A	6	333.77	5.57
FiLL097_2012_2014_3	FiLL113_2010_2015_3	3	148.53	420.63
FiLL012_2007_2008_A	FiLL066_2012_2012_A	5	35.61	19.58

is capable of dispersing long distances, for example up to 215 km females and to 428 km males (Samelius et al., 2011), thus potentially allowing for strong gene flow across large areas and among subpopulations (Wayne & Koepfli, 1996) when populations persist through the landscape. In support of this, Ratkiewicz et al. (2014) found evidence of active gene transfer between Finnish and Russian lynx populations. Thus, overall it seems likely that the genetic clustering observed in our study is a signature of the many different pairs of close relatives identified within each cluster, but not between them.

The observed significant correlation of genetic and geographic distances, that is the shorter the distance between individuals, the higher the pairwise relatedness, further corroborates this interpretation. Some of the individuals not clearly assigned to clusters and thus characterized as admixed genotypes, had close relatives among those individuals assigned to a genetic cluster. This indicates mixing of the genetic groups and that those individuals had the same mother but probably a different father. Another possible explanation is that the admixed individuals originate from family groups located outside the study area.

The median distance between all females in our study was over four times longer than between siblings (55.6 km) and over six times more than between parent and offspring (36.8 km). There was a large variation in distances especially for siblings, whereas most

TABLE 4 Recognized 38 sibling relationships (60 individuals) in a lynx population in southern Finland during years 2007–2015. The likelihood ratios (LR) above 20 were sorted from the highest to the lowest. Individual identification contains ID-number, year of birth, year of death, and genetic cluster (1, 2, 3, and A = admixed)

Individual 1	Individual 2	Age difference	LR	Distance (km)
FiLL019_2007_2009_3	FiLL039_2008_2011_3	1	4,583,100.00	31.29
FiLL012_2007_2008_A	FiLL068_2008_2012_A	1	468,167.00	25.35
FiLL054_2011_2012_3	FiLL098_2010_2014_3	1	126,768.00	4.47
FiLL083_2011_2013_2	FiLL089_2010_2014_2	1	111,193.00	24.26
FiLL012_2007_2008_A	FiLL038_2008_2010_1	1	102,532.00	31.47
FiLL129_2011_2015_3	FiLL131_2012_2015_3	1	65,264.70	226.12
FiLL111_2012_2015_A	FiLL120_2013_2014_A	1	42,453.00	131.72
FiLL036_2003_2010_1	FiLL130_2004_2015_1	1	37,306.90	345.29
FiLL038_2008_2010_1	FiLL068_2008_2012_A	0	21,747.40	6.35
FiLL060_2008_2012_2	FiLL071_2009_2012_2	1	12,468.80	15.59
FiLL006_2006_2007_2	FiLL067_2006_2012_2	0	9,763.53	49.72
FiLL115_2014_2014_A	FiLL118_2014_2014_1	0	7,762.84	57.53
FiLL008_2005_2008_1	FiLL115_2014_2014_A	9	6,694.10	76.45
FiLL093_2012_2014_1	FiLL106_2012_2014_1	0	3,286.93	207.87
FiLL023_2010_2010_2	FiLL103_2010_2014_2	0	2,617.23	27.54
FiLL011_1994_2007_A	FiLL119_2005_2015_3	11	1,524.69	121.27
FiLL029_2008_2011_1	FiLL114_2013_2015_1	5	1,305.12	521.55
FiLL006_2006_2007_2	FiLL023_2010_2010_2	4	575.96	18.38
FiLL094_2013_2014_1	FiLL123_2014_2015_1	1	424.58	180.03
FiLL056_2011_2012_A	FiLL079_2011_2012_3	0	364.74	15.59
FiLL034_2010_2011_2	FiLL075_2000_2013_2	10	212.87	64.24
FiLL080_2010_2012_3	FiLL129_2011_2015_3	1	173.58	237.91
FiLL016_2006_2008_3	FiLL039_2008_2011_3	2	166.84	47.49
FiLL044_2011_2012_3	FiLL084_2013_2013_3	2	153.13	10.63
FiLL008_2005_2008_1	FiLL127_2014_2014_1	9	106.61	347.79
FiLL021_2008_2010_A	FiLL125_2011_2015_A	3	100.57	32.55
FiLL016_2006_2008_3	FiLL101_2013_2014_3	7	75.83	39.92
FiLL034_2010_2011_2	FiLL060_2008_2012_2	2	69.16	55.62
FiLL002_2006_2007_1	FiLL057_2006_2012_1	0	58.63	39.34
FiLL063_2011_2012_3	FiLL097_2012_2014_3	1	54.92	16.93
FiLL044_2011_2012_3	FiLL078_2009_2013_3	2	53.37	44.52
FiLL035_2009_2010_1	FiLL050_2010_2012_1	1	43.95	29.94
FiLL106_2012_2014_1	FiLL132_2011_2015_A	1	39.94	147.95
FiLL036_2003_2010_1	FiLL091_2012_2014_1	9	33.74	67.80
FiLL071_2009_2012_2	FiLL089_2010_2014_2	1	26.79	63.53
FiLL027_2009_2010_3	FiLL048_2009_2012_A	0	25.69	153.13
FiLL061_2011_2012_3	FiLL131_2012_2015_3	1	23.30	135.42
FiLL002_2006_2007_1	FiLL104_2009_2014_A	3	23.25	85.08

mother–daughter pairs were relatively close (although some outliers were found). The cost of defending a given territory may increase with population density, resulting in increased home-range overlap (Rodgers et al., 2015). Under these conditions, it may be that related individuals tolerate the costs of sharing resources due to benefits gained from inclusive fitness (Anderson, 1989). Kin clusters that are associated with home-range overlap could potentially support higher local population densities also for Eurasian lynx. However, a strong kin cluster in an area may also potentially hinder immigrating unrelated female lynx from establishing new territories in the vicinity. Immigration by many species of territorial mammals and birds appears to be limited by crowding (Lambin, Aars, & Piertney, 2001).

TABLE 5 Recognized 26 pairs of Mother–offspring and siblings in a lynx population in southern Finland during years 2007–2015. The likelihood ratios (LR) above 20 were sorted from the highest to the lowest. Individual identification contains ID-number, year of birth, year of death, and genetic cluster (1, 2, 3, and A = admixed)

Individual 1	Individual 2	Age difference	LR Mother–offspring	LR Siblings	Distance (km)
FiLL060_2008_2012_2	FiLL075_2000_2013_2	8	4,556,980.00	829,753.00	11.99
FiLL004_2005_2007_1	FiLL041_2000_2011_1	5	2,335,580.00	1,649,680.00	35.17
FiLL044_2011_2012_3	FiLL086_2006_2013_3	5	650,720.00	19,892.80	13.49
FiLL071_2009_2012_2	FiLL075_2000_2013_2	9	470,696.00	2,092.31	13.32
FiLL084_2013_2013_3	FiLL086_2006_2013_3	7	232,597.00	18,472.40	3.49
FiLL091_2012_2014_1	FiLL130_2004_2015_1	8	151,655.00	5,097.38	278.58
FiLL007_1997_2008_1	FiLL053_2006_2012_1	9	144,159.00	6,033.54	59.38
FiLL007_1997_2008_1	FiLL099_2000_2014_1	3	110,972.00	1,577.43	17.65
FiLL051_2009_2012_1	FiLL058_2012_2012_1	2	106,152.00	606.02	19.24
FiLL060_2008_2012_2	FiLL112_2011_2014_2	3	101,015.00	2,889.34	534.65
FiLL049_2004_2012_3	FiLL074_2010_2013_3	6	63,841.50	61,919.40	43.93
FiLL013_2004_2008_A	FiLL045_2002_2012_A	2	21,391.80	85.02	69.01
FiLL091_2012_2014_1	FiLL127_2014_2014_1	2	13,378.30	469.62	272.93
FiLL034_2010_2011_2	FiLL067_2006_2012_2	4	11,748.10	28.47	6.74
FiLL001_2004_2006_A	FiLL031_2000_2011_1	4	7,866.37	210.61	69.92
FiLL101_2013_2014_3	FiLL113_2009_2015_3	4	5,882.27	208.03	460.77
FiLL016_2006_2008_3	FiLL113_2009_2015_3	4	3,574.93	385.07	421.46
FiLL011_1994_2007_A	FiLL017_2006_2009_A	12	3,547.81	214.95	9.38
FiLL004_2005_2007_1	FiLL050_2010_2012_1	5	3,531.88	277.37	56.99
FiLL045_2002_2012_A	FiLL059_2011_2012_1	9	3,259.75	92.53	1.51
FiLL049_2004_2012_3	FiLL088_2011_2013_3	7	2,382.41	576.59	33.71
FiLL040_2009_2011_A	FiLL076_2011_2013_A	2	2,245.33	490.37	62.05
FiLL008_2005_2008_1	FiLL118_2014_2014_1	9	1,712.52	159.55	19.45
FiLL046_2002_2012_A	FiLL125_2011_2015_A	9	1,662.39	161.59	17.93
FiLL063_2011_2012_3	FiLL101_2013_2014_3	2	1,008.62	266.81	35.47
FiLL066_2012_2012_A	FiLL068_2008_2012_A	4	961.77	56.44	9.39

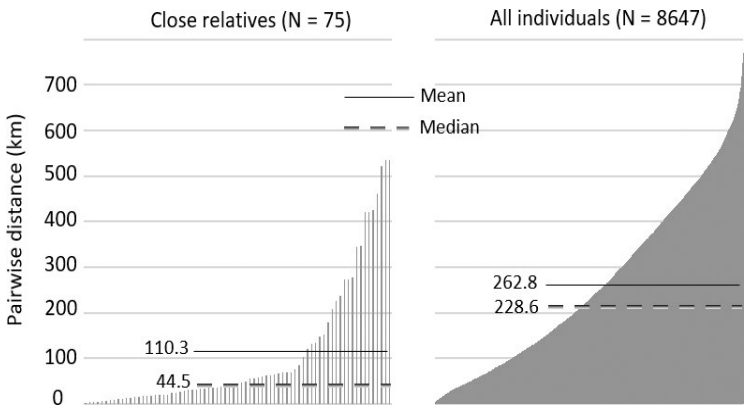

FIGURE 6 Values for median and mean pairwise geographic distances (km) between individuals of close relatives according to genetic relatedness and all individuals pooled. Median value = dashed line; Mean value = solid line; *N* = 132

Inversely density-dependent dispersal, impeding both immigration and emigration, seems to be true for Eurasian lynx in fragmented populations (Zimmermann, 2004; Zimmermann, Breitenmoser-Würsten, & Breitenmoser, 2005, 2007), but whether or not this is also the case in unfragmented ones, such as in Finland, remains to be investigated.

Our results support the hypothesis that Eurasian lynx form matrilineal assemblages at regional scale. However, Elbroch et al. (2015)

found mixed support for the existence of cougar matrilineal lines in the southern Yellowstone. Some resident females immigrated into the study area from elsewhere, even while the pedigree revealed several clear matrilineal lines and even some philopatric males. For leopards, when species density increased after decreased harvest pressure, females formed matrilineal kin clusters, suggesting substantial negative effects of harvest disturbances on population size and social structure (Fattebert et al., 2016).

When studying social organization based on genetic data, special attention should be given in selecting the right combination of methods, taking into account species, population size, dispersal capability, the degree of population fragmentation, and the spatial and temporal extent of the study. These may be the reasons why a previous study on a small, isolated population showed generally lack of a relationship between the spatial distance and relatedness among individuals, but on the other hand, showed the domination of the entire population by a limited number of reproducing individuals, which partly indicates kin clustering (Schmidt et al, 2016). It could also mean that social structure is flexible and changes with external conditions. Philopatry, however, does not necessarily lead to genetic clustering (Biek et al., 2006). Biek et al. (2006) found that even though female pumas remained philopatric, there was no genetic clustering, although genetic legacy of females with high reproduction success could be traced. They assumed that either the females were not successful in leaving philopatric offspring or the males, which emigrated from more distant populations, brought enough different alleles to outweigh the clustering phenomenon. It is also worthwhile to ask, is genetic clustering of related individuals always a proof of philopatry? The question relates to the ecologically meaningful spatial scale of the species and the scale used in the study. In species with a social organization determined by family bonds, the spatial distribution of related individuals and the level of genetic clustering we observed might potentially be higher in small, isolated, and fragmented populations as a result of lower dispersal possibilities. Indeed, subadults female lynx may be less prone to cross barriers such as highways and densely populated valleys than males (Zimmermann, Breitenmoser-Würsten, & Breitenmoser, 2005, 2007). However, the opposite effect is also not unthinkable based on the inversely density-dependent dispersal behavior of lynx (Zimmermann, Breitenmoser-Würsten, & Breitenmoser, 2005, 2007) and, moreover, the possibility that matrilineal structure might disappear at low population densities, as observed for leopard (Fattebert et al., 2016).

The whole Finnish lynx population is of native origin and historically, it experienced periods of population decline. However, it is continuous and well connected to the population in Russia via extensive woodlands (Chapron et al., 2014). As such, our study contributes reference values for genetic parameters from a large lynx population in an almost unfragmented habitat. Robust reference values from large lynx populations are required for the assessment of the genetic status, management, and remedy of the still small reintroduced lynx populations in central Europe (e.g., Zimmermann, 2004; Zimmermann, Breitenmoser-Würsten, & Breitenmoser, 2005; Zimmermann, Breitenmoser-Würsten, & Breitenmoser, 2007). Whether the patterns observed among female lynx in our study represent true matrilineal assemblages will be further clarified by including males in analysis. This will also lead to obtaining estimates for the genetic parameters of the whole population. Studies including also males are now needed to further assess the social organization of this species.

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CONFLICT OF INTEREST

None declared.

AUTHOR CONTRIBUTIONS

KH, SBH, AH, AK, JS, and HGE designed the study. KH was responsible for collected samples. JS generated the raw data in the laboratory. AK, SBH, and JS analyzed the data. All authors contributed to the writing of the manuscript. All authors approved the final version of the manuscript.

DATA ACCESSIBILITY

Data for this study are available at Dryad Digital Repository: https://doi.org/10.5061/dryad.7pc6ms3 Data files: Holmala_lynx_kinclusters.

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